ALLOXURIC EXCRETION IN A CASE OF LEUCOPENIA.

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INTRODUCTORY.

It is now nearly half a century since it was first pointed out by H. Ranke[1] that the excretion of uric acid is increased in certain cases of leucocythæmia. Since then nearly fifty independent workers have repeated the investigations, and with few exceptions have corroborated Ranke's observation. Amongst those who obtained a contrary result may be mentioned Mosler[2] and Jacubasch,[3] but it must be remarked that in the cases investigated by these observers other factors presented themselves which might be held accountable for the absence of increase. Thus in Mosler's case there was very marked debility with a correspondingly low nitrogen excretion, whilst in Jacubasch's patient the existence of œdema and diarrhœa may account for the exceptional result.

In most of these observations, however, the method employed to estimate the uric acid was that of Heintz, which has since been shown to be far from correct. Since the introduction of the more exact methods of Hopkins, Salkowski and Ludwig the number of cases examined has not been great, and of these only a few are suitable for comparison with the physiological excretion, the composition of the diet having been left out of account in the majority of cases.

[1] Beobachtungen und Versuche über die Ausscheidung der Harnsäure beim Menschen. München, 1858.

[2] Virchow's *Archiv*, 1866, xxxvii, p. 45.

[3] Virchow's *Archiv*, 1868, xliii, p. 217.

Where both these sources of inaccuracy have been eliminated, the general result of the investigations has been to show that an increase in the number of leucocytes does not necessarily go hand in hand with a rise of endogenous alloxuric bodies in the urine, but that only in cases of spleno-medullary leucocythæmia is a distinct increase present.[4] On the other hand, the lymphatic form of the disease seems to show no increase, whilst leucocytoses, whether physiological[5] or produced by the administration of drugs,[6] are devoid of any constant effect. It would therefore seem that Horbaczewski's theory, which connects an increase of alloxur bodies with a corresponding leucocytosis, does not hold good for all forms of leucocytic increase.[7]

Investigations to decide the question of a parallelism between the number of leucocytes and the alloxuric excretion have usually been carried out on cases of leucocythæmia, whereas very few observations have been recorded in cases in which the number of leucocytes was diminished.

In the following case, where a distinct leucopenia was present, we estimated the amount of alloxuric nitrogen excreted in the urine each day during two periods of eight days each. In doing this consideration was taken of the valuable work of Burian and Schur,[8] who pointed out that, before any estimate could be formed of the amount of the bodies excreted, a careful regulation of the diet was necessary in order to be certain that endogenous purins only were excreted, for it is in them that any increase or decrease is of importance, since they alone arise from the metabolic processes in the tissues.

CLINICAL HISTORY.

Mrs. B., aged 37, a widow, was admitted to the London Hospital in the beginning of June, 1900, complaining of "general weakness." Her ill-

[4] Milroy and Malcolm, *Journal of Physiology*, 1898, xxiii, p. 235, and 1899, xxv, p. 109.

[5] Sivén, *Skandinav. Arch. f. Physiologie*, 1900, xi, p. 123, and Kühnau and Weiss, *Zeitschr. f. klin. Med.*, 1897, xxxii, p. 482.

[6] von Noorden and Zuntz, DuBois-Reymond's *Archiv, Physiol. Abth.*, 1894, p. 203.

[7] For details see Schreiber, Ueber die Harnsäure unter physiol. u. pathol. Bedingungen. Stuttgart, 1899.

[8] *Arch. f. d. gesammte Physiologie*, 1900, lxxx, p. 241.

ness had begun about twelve months before admission immediately after she left Gibraltar, where she had been living for about a year. Previous to this she had always enjoyed good health. There was no evidence of her having suffered from either syphilis or malaria. She was a poorly nourished, fair woman (weight 7 stones), pale and of somewhat yellowish complexion.

There was no jaundice, cyanosis nor dropsy. There were a few purpuric spots round the ankles, but no other evidence of hæmorrhage either in the skin or retina.

The spleen was enormously enlarged, extending down beyond the umbilicus into the right iliac fossa. The edge was rounded; the surface somewhat irregular and slightly tender. The liver was also enlarged, the lower border being three inches below the costal margin in the mammary line. Its surface was smooth and firm. There was no glandular enlargement and no ascites. No abnormality was detected in the thoracic organs, nervous system or urine.

The blood contained 4½ million red corpuscles, 65 per cent of hæmoglobin, and 1500 to 3000 white corpuscles. The lymphocytes and polynuclears were practically equal in number. No eosinophiles and no abnormal elements were seen in several stained films.

The temperature during her stay in the hospital was persistently irregular, usually running up to 103° F. or 104° F. in the evening. The pyrexia exhibited a tendency to occur in periods of 10 or 12 days, separated by 2 or 3 days of almost normal temperature. She had occasional bleedings from the nose and a tendency to diarrhœa, but ate well and maintained the same weight throughout.

At first the case was regarded as one of splenic anæmia and was treated on that assumption without improvement. The fact, however, that her illness had begun shortly after leaving Gibraltar aroused the suspicion that she might be suffering from Malta fever. The reaction of her serum to Micrococcus melitensis (Bruce) was accordingly tested by Dr. Bulloch. The serum was found to agglutinate the cocci in the proportion of one part of serum to 30 of culture, and in accordance with this the conclusion was arrived at that the case was one of Malta fever. The patient remained in hospital until the end of 1900 without exhibiting any marked change in her condition, though the leucocytes had increased to about 3000 per cubic millimetre under treatment with nuclein. From a clinical

point of view the case was remarkable as exhibiting an absence of the joint pains usually present in Malta fever and the presence of a much greater degree of splenic enlargement and of leucopenia than are usually found in that disease.

METHODS.

During both periods the patient was confined to bed, and was kept on a diet consisting of eggs, milk, bread and farinaceous foods, but containing no flesh nor other purin-yielding ingredients. The exact amount of food consumed was ascertained, and the urine was collected for each day. The fæces were neglected, since no appreciable amount of purins is lost with them.

The total nitrogen was estimated by Kjeldahl's method, and the nitrogen of the total alloxuric bodies by that of Camerer. Parallel estimations were made in each sample during the second, and in most of those of the first period.

RESULTS.

TABLE I.—1ST PERIOD.

Date.	N. of Food*.	Urine Amount.	Total N.	Alloxuric N.
July 2	16 grm.	812 cc.	9.630 grm.	0.1867 grm.
3	14	1315	6.996	0.1330
4	14.5	1475	8.152	0.1652
5	17	1350	11.1321	0.2295
7	16	1965	10.779	0.1224
8	16	1750	11.2000	0.1224
9	15	1940	14.550	0.2211
10	15	1965	12.3402	0.1670

* Calculated from König's tables.

During this experiment the patient received approximately about 15 grammes of nitrogen in the diet, but this amount varied somewhat and was accompanied by corresponding swings in the urinary nitrogen. It would seem as if a considerable nitrogen retention was taking place in the tissues, but the patient had several attacks of diarrhœa during the investigation, whereby some nitrogen may have been lost. The total alloxuric nitrogen averaged about .17 grm. in the 24 hours, but was on two occasions as high as .22 grm., and on another as low as .122 grm. Such variations in the endogenous alloxuric excretion have been denied by Burian and Schur to exist physiologically, but, as these observers point out, they may occur in disease. During the

period of examination, also, it is probable that, on several occasions, a certain amount of urine was lost with the fæces. The average, however—namely, .17 grm.—falls within the limits of the physiological excretion, this varying between .122 grm. and .20 grm.

TABLE II.—2D PERIOD.

Date.	N. of food.	Urine Amount.	Total N.	Alloxuric N.	Leucocytes.	S. P*.
Sept. 4	17.2 grm.	1140 cc.	13.23 grm.	0.2042 grm.	2184	1 : 1
5	18	1695	15.36	0.2084		
6	18	1560	12.036	0.2085	1560	3 : 2
7	18	1465	10.814	0.1871	....	
8	17.5	1205	10.567	.....	1872	2 : 3
9	18.5	1365	12.558	0.2064	3000	5 : 4
10	19	1100	13.530	0.2002		1 : 1
11	19	1570	14.750	0.2103		11 : 10

*S. signifies simple nucleated leucocytes; *P.* signifies polymorphonuclear leucocytes.

The investigation recorded in Table II was carried out about two months after the previous one, and during it the patient's general condition was much better, there being no diarrhœa. The urine was collected with greater care, and only on one occasion (namely, on Sept. 8) was there any admixture with fæces.

The diet was free from alloxur bodies and contained rather more nitrogen than on the previous occasion. The total alloxuric nitrogen showed only slight variations, its average being 0.20 gr. in the 24 hours. This is considerably more than during the previous period, which may be accounted for by the improved condition of the patient and the more careful investigation of the case. It still falls within the normal for alloxuric nitrogen however, although at the highest limit of this.

CONSIDERATION OF RESULTS.

The average number of leucocytes per cubic millimetre during the two periods was 2500, sometimes reaching as high as 3000 or falling to 1800. Regarding their nature, the most important point is the relative increase of lymphocytes, or, more correctly stated, the decrease in the total leucocytes affected the polymorphonuclear cells to a greater extent than the lymphocytes. This great diminution in the granular cells might conceivably be due to one of two causes:

1. To an increased destruction, the production remaining normal.

2. To a diminished production, the rate of destruction remaining normal.

If the former of these conditions were present, the amount of disintegration products of the leucocytes excreted by the urine—*i. e.*, of alloxuric bodies and of phosphorus—would be increased. If the latter obtains a diminution in these would be expected. In the case before us, however, the excretion falls within the normal, though at the highest limit of this; and if one considers that the patient weighed only 45 kg., and had probably a low endogenous factor, then it may be assumed that a slight increase was present, and therefore that the leucopenia was occasioned by active destruction of the polynuclears in the enlarged spleen, and that the red bone-marrow had not been able to make good the loss. It would seem as if in this regard the only difference between this case and one of spleno-medullary leucocythæmia was the bone-marrow activity. In leucocythæmia this organ is more active than normal, thus making good the increased destruction by the spleen and even overcompensating it, whereas in leucopenia it has not compensated for the splenic destruction.[9]

Another clue to the amount of leucolysis occurring in the body is, as Milroy and Malcolm[10] have pointed out, the amount of phosphorus excreted. In ascertaining this, however, the ingested phosphorus must be very carefully estimated, which fact introduces serious difficulties when we come to apply the method to patients. On four days following the second period we estimated the total phosphorus excreted with the following results:

TABLE III.

Date.	Urine Amount.	Total P_2O_5*.
Sept. 18	1100 cc.	2.266 grm.
19	1570	2.386
20	1220	2.170
21	1300	2.418

* Estimated by titration with uranium nitrate solution, using tincture of cochineal as indicator.

[9] In this connection, however, it should be pointed out that it is conceivable that both formative and destructive organs may act on a high or on a low level. In the former case the number of leucocytes might remain constant, but their life history be of short duration, and the alloxuric excretion consequently increased, whereas, in the latter case, where both organs were depressed in activity, and the life history of the leucocytes of long duration, a normal number of leucocytes would be accompanied by a diminished alloxuric excretion.

[10] *Journal of Physiology*, 1898, xxiii, p. 217, and ibid., 1899, xxv, p. 105.

The diet was similar to that during the previous examinations, and was of exactly the same amount each day, containing probably a little less phosphorus than an ordinary mixed diet. From this it would appear that, if anything, the excretion of phosphorus was slightly increased, pointing to an increased leucolysis.

A consideration of these different points renders it probable that the leucopenia in this case was due to an inability on the part of the red bone-marrow to make good an increased leucolysis in the spleen. The entire absence of any myelocytes or other abnormal blood-cells would seem to support this opinion.

SUMMARY.

1. In the case investigated, one of Malta fever, the leucocytes were reduced to between 1500 and 3000 per cubic millimetre.

2. Notwithstanding this, the alloxur bodies and the phosphoric acid in the urine, the patient being on an alloxur-free diet, showed no distinct diminution from the normal.

3. The suggestion is made that this result may be due to the leucopenia being brought about by an increased destruction of leucocytes in the spleen rather than to a diminished activity of the bone-marrow.

STUDIES ON THE MORPHOLOGY OF GANGLION CELLS IN THE RABBIT.

I. The Normal Nerve Cells.
II. Changes in the Nerve Cells in Rabies.

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Plates XXXIV–XXXVIII.

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I.—THE NORMAL NERVE CELLS.

Plates XXXIV and XXXV.

INTRODUCTION.

The purpose of these investigations was the study of the changes which take place in the nerve cell as the result of disease, and especi-

ally the sequential order of these changes from their inception to their termination. With this end in view, the plan was to take up in turn the study of the sequence of nerve-cell changes in a number of experimentally produced diseases; the aim being to determine if possible, by a comparison of the results in the different diseases, either a specific type of cellular changes or sequence of changes for each separate morbid process, or a unity in the effects of different morbid conditions upon the nerve cell, and thus to deduce, if possible, some general laws governing the sequence of morbid changes.

The advantages, in fact the necessity of using animals and experimental methods in such a study, are obvious. Aside from the ability to obtain material at pleasure from any desired stage of a disease, is the advantage of completely eliminating post-mortem changes, which are an almost constant possible source of error in material obtained from human autopsies. The animals used were the rabbit and the guinea-pig.* These have the advantage, on the one hand, of being such common subjects of experimental inoculation that their reactions to the various bacteria and their toxins are comparatively well known, thus rendering unnecessary extensive control series, and on the other hand, of approximating sufficiently in organization to man to warrant some attempt at least at analogical deductions.

At the very outset a complete barrier to accurate pathological work was encountered in the lack of satisfactory data, and especially of accurate drawings of the nerve cells of the normal rabbit and guinea-pig. In studies such as the present, involving investigations of cytological changes, an extremely accurate norm of comparison is essential. Originally intended merely as a necessary preliminary to the pathological work, this study of the normal cell has constantly increased in scope and importance until it now makes a large part of the work. Yet if it may furnish to other investigators in this field of experimental pathology a basis for comparison and eliminate for them

* I am at present engaged in work upon the nerve cells of the normal guinea-pig and upon the changes in guinea-pigs subjected to the tetanus toxin, and to the diphtheria toxin; and also upon the changes in the nerve cells of rabbits inoculated with the poisons of the rattlesnake and of Heloderma. See preliminary report before the New York Academy of Medicine in the *Medical Record*, (New York), June 2, 1900.

the necessity of going over the normal, it may be of service. At the same time, throughout the studies in normal cytology, the original purpose has been kept clearly in view. Thus there has been no attempt at a study of all the nerve cells of the normal rabbit and guinea-pig. Only the cells from certain definitely selected regions of the normal central nervous system have been selected for study in order that they might furnish a basis for comparison with cells from the same regions of probably pathological nervous systems in the same animals.

The first part of this paper is devoted to a description of normal nerve cells of the rabbit, and the second part (p. 581) to a consideration of the changes in these cells in rabies.

HISTORICAL REVIEW.

Before presenting the results of our study of the structure of the nerve cell in the normal rabbit, it is desirable to review briefly the present state of our knowledge concerning the internal structure of the nerve cell in general. A full presentation of the existing knowledge upon this subject is contained in Barker's admirable book "The Nervous System and its Constituent Neurones" (31).

So profound an influence had the introduction of Nissl's methods of staining nerve cells, the first of these published in 1885 (1), that it is convenient to divide the history of the development of our knowledge of the internal structure of this cell into the periods before Nissl and after Nissl. In the earlier period the majority of investigators described the internal structure of the nerve cell as fibrillar in character. Between the fibrils was a ground or basement substance, homogeneous or finely granular, about which little was said. Opposed to this early idea of a fibrillar structure of nerve cells was a smaller group of investigators, among them Key and Retzius (2), and Arndt (3), who described the protoplasm of the cell as homogeneous or granular, with larger masses scattered through it; to an arrangement of these granules in rows or threads they attributed the fibrillar appearance noted by other writers.

The principles of Nissl's method are extremely simple. Briefly, they consist in the use of a quick fixative, such as alcohol, and subsequent staining with one of the basic aniline dyes, such as methylene blue. Nissl recognizes in the structure of nerve-cell protoplasm stained by

his method (certain variable pigmented deposits excepted) two distinct substances: 1, a stainable substance, which reacts strongly to basic aniline dyes; and 2, a non-stainable substance, i. e. one which fails to react to basic aniline dyes.

Based upon this reaction and upon variations in arrangement, which the stainable substance presents in different cells, Nissl (4) has formulated a system of classification of nerve cells as follows:

1. Cells which react only as to their nuclei to the basic anilines, the cell body remaining unstained. Of these cells, Nissl recognizes two subgroups:

(a) Cytochromes. These are the cells formerly spoken of as "granules," those of the molecular layer of the cerebellum being an example. The nucleus is no larger than that of the neuroglia cell.

(b) Caryochromes. The cell body is usually free from chromatin and consequently unstained. Rarely a small bit of stainable substance is found to one side of the nucleus. The nucleus resembles that of other nerve cells, and is larger than that of neuroglia cells.

In neither of these cells is there by this method any definitely outlined cell body, and yet that they have very definite cell bodies, and that to at least one other stain these bodies present the same reaction as do those of other nerve cells, has been shown by the method of Golgi.

2. Cells which react both as to their nuclei and as to their cell bodies to the Nissl stain. To these cells Nissl has given the name "somatochromes." He recognizes three subgroups:

(a). Arkyochromes or cells in which the chromatic substance is present in the form of anastomosing chains, making a coarser or finer meshwork; as, for example, in the Purkinje cells of the cerebellum and in the mitral cells of the olfactory lobe. Again subdividing, he describes two types of arkyochrome cells:

(α). Enarkyochromes or cells in which the nodal points of the network are united by fine threads of chromatic substance.

(β). Ampharkyochromes or cells in which the nodal points are united by heavier bands or bridges which take a dense stain.

(b). Stichochromes or cells in which the chromatic substance is arranged in more or less distinctly parallel rows, the direction of the rows usually bearing some relation to the contour of the nucleus and to that of the periphery of the cell body. To this group belong such cells as the large cells of the ventral horn of the cord, the spinal-ganglion cells, some of the cells of the cornu Ammonis, and some of the cells of the cerebral cortex.

(c). Gryochromes or cells in which the chromatic substance is present

in the form of fine granules. Such cells Nissl describes in the corpus striatum.

This classification Nissl at first believed to be of both morphological and physiological significance; i. e. that each morphological type of cell corresponds to a physiological function. While this appears to be true within certain narrow limits, the classification is now accepted mainly in a morphological sense. The importance of this classification in the present instance is that Nissl claims, and is supported therein by many other investigators, that these types are perfectly definite, not only for man but also for lower animals; that a cell of a given type found in a given region in the human nervous system, will be found to occur in precisely the same or analogous region as we pass downward through the animal series. Thus in studying cells from a particular locality in the nervous system of an animal, we should expect to find a correspondence in type to cells found in the same or analogous localities in man and in other animals, the differences being only within the range of variation of that type.

Again recognizing a variation in staining reaction among cells of the same type—a variation quite constantly found, and believed to be explicable by chemical changes dependent upon the metabolic condition of the cell—Nissl has classified cells of the same kind according to their different chromatic reactions. In some cells the chromatic elements are closely packed and the cell consequently takes a dense stain; these he describes as in the pyknomorphous condition. In others, the chromatic elements are more loosely distributed, and these cells, staining less intensely, are said to be in the apyknomorphous condition. A mean condition between these two is designated as parapyknomorphous. A condition in which the entire cell body stains intensely, there being apparently, little or no non-stainable element present, is designated by Nissl as chromophilic. The significance of these cells is not clear. Their occurrence seems to be more common after some fixatives than after others, and they are said to be more frequent in portions of the tissue exposed to the direct action of the reagent. Opinion tends to their interpretation as artefacts.

The physical and the chemical nature of this stainable element of the nerve cell, or more properly, of that element of the cell body which presents such a definite reaction to the Nissl stain, still furnishes a wide field for investigation and discussion. Von Lenhossék (5), Held (6), de Quervain (7) agree that this substance, as seen in sections of hardened material, is, however homogeneous in appearance, really granular in character. Following up this idea, Held and von Lenhossék inves-

tigated the conditions in fresh material. The latter, in spinal-ganglion cells, freshly teased in an indifferent medium, could see, in addition to the nuclear structure, only a finely granular protoplasm, without definite structure or arrangement. Held's investigations, while corroborating those of von Lenhossék, went farther. Fresh nerve cells, examined immediately after removal in a passive medium, showed distinctly outlined nuclei with nucleoli, but in the protoplasm of the cell bodies no formed elements whatever. These remained perfectly clear and homogeneous, or slightly granular, depending largely upon rapidity of technique, being the more clear, the sooner after removal the cells were examined. With methylene blue, in dilute solutions, he obtained a reaction showing granular masses, even in cells so fresh as to show no structure when examined unstained. He considers this due to a fixative action of the methylene blue. By subjecting these fresh cells to the action of the various fixing agents commonly used in the Nissl technique, Held claims to have observed in his previously clear protoplasm the formation of granular masses, which he considers identical with the Nissl bodies as revealed by the usual Nissl procedure. In sections of hardened material, stained blue with erythrosin and methylene blue, he finds the Nissl granules embedded in a homogeneous coagulum-like mass, which stains violet in contra-distinction to the blue of the granules and to the red of the acidophile elements of the cell.

Investigations have been made also by Held, Halliburton (8), Macallum (9), and others upon the chemistry of the Nissl bodies with the following general results: They are soluble in alkaline fluids of all strengths, and insoluble in acids, ether, and alcohol. They respond to tests for phosphorus and, according to Macallum, to tests for iron. As a result of his investigations, both histological and chemical, Held concludes that in the Nissl bodies we are dealing with a phospho-albumin, similar to the nucleo-albumins; that in the living cell this phospho-albumin exists in solution among the other ingredients of the protoplasm; that the action of fixatives is to cause a coagulation or precipitation of the albuminoid substance and in this way to give rise to the Nissl picture. Opposed to this view are the findings reported by Flemming of masses representing Nissl bodies in fresh cells.

As to the structure of that portion of the cell body which does not react to the Nissl stain, three main theories, each founded upon the studies of its originator, and supported with certain minor modifications by what may be termed his "school," exist:

1. That the structure is fibrillar in character. Originating with Remak (10) and Max Schultze (11) long before the introduction of the

Nissl ideas, it has been supported by Ranvier (27), Boll (28), Schwalbe (29), Kronthal (12), Dogiel (13), in a modified form by Flemming (14) and E. Müller (15), and more recently by Bethe (16), Becker (17) and Apáthy (18). That all of these believers in the fibrillar structure of what we may call the ground substance of the nerve cell, are in agreement as to the structure and arrangement of these fibrillæ is far from true. The early supporters of this doctrine looked upon the nerve cell as essentially fibrillar, the fibrils being merely held together by a web of interfibrillar cement. Bethe and Apáthy, among the recent investigators, strongly support the fibrillar theory. Bethe describes minutely the course of the fibrils entering and leaving the cell by its various processes, the fibrils seeming to pass through the cell body, rather than to be an integral part of its organization. Apáthy's ideas of both the intra-cellular and the extra-cellular courses of the fibres are even more complex than are Bethe's. Both as yet need confirmation.

2. Bütschli (30), extending to nerve cells his theories concerning the foam-like structure of protoplasm in general, sees in these cells a honeycomb structure, the whole cell body being made up of a great number of superimposed elementary cells or compartments after the fashion of a honeycomb. He explains the discrepancies in the results of other investigators as largely due to the different pictures presented by these structures, depending upon thickness and upon direction of the plane of section. In some of his most recent writings, Held seems to subscribe, with certain reservations, to the theory of Bütschli. He believes, however, that these honeycomb structures represent artefacts of fixation, rather than structures preexistent in the protoplasm of the living cell.

3. The idea of a distinct reticular structure within the non-stainable substance of Nissl receives the support of Ramon y Cajal (19), Held (20), van Gehuchten (21), Van Gieson (22), and Ewing (23); Held, as already stated, accepting Bütschli's theory as a possible explanation of the reticular appearance. This reticulum traverses with its strands a more fluid element, the cytolymph or cytoplasm. Held describes in very thin sections, stained with erythrosin and methylene blue, a granular appearance of this reticulum, and in the axis-cylinder process an arrangement of these granules in rows, giving the appearance of striations or fibrillæ.

Special attention has been called by Rosin (24), to the micro-chemical differentiation of the constituents of the cell body, the Nissl bodies reacting to the basic dyes, while the ground substance is acidophilic in character.

METHODS.

Material.—For the study of normal cells, six rabbits were used. Those were selected which had been for some time under observation, none having been previously used for experimental purposes, or having shown any sign of disease. All were perfectly healthy normal rabbits. Rabbits of medium weight were chosen without reference to sex. The internal organs were normal.

Methods of Killing.—Rabbits Nos. 1 and 2 were killed by chloroform in an air-tight jar of about ten litres, so that beyond the amount originally in the jar, no air was admitted with the chloroform; there may therefore have been some asphyxiation. These rabbits were unconscious at the end of three minutes, when dissection was begun.

Rabbit No. 3 was killed by chloroform in the open air, dying in about five minutes.

Rabbit No. 4, killed by ether in a jar partially air-tight, died in about six minutes.

Rabbit No. 5, killed by cutting the throat, bled to death almost instantly.

Rabbit No. 6 was killed instantly by a blow on the back of the neck.

From the standpoint of convenience, the method of placing the animal in an air-tight jar with sufficient chloroform to kill it, is the most satisfactory. The other methods served as controls, it seeming easily within the range of possibility, that certain cells, as, for example, those of the olfactory lobe might present modifications in their finer structure due to the action of such irritant substances as ether, chloroform, or carbonic-acid gas. It was with this possibility especially in view that the methods adopted for rabbits 5 and 6 were selected.

Comparative studies upon the effects on the finer structures of the nerve cell of these different methods of killing showed that no specific effects—at least none demonstrable by the Nissl technique—were produced by the different modes of death used. Cells from rabbits killed by the different methods and then subjected to the same technical procedures, presented an extremely uniform appearance.

Fixation and Hardening.—In all cases the brain, cord and spinal ganglia were removed as quickly and as carefully as possible, cut transversely into thin slices by a sharp razor wet with normal saline solution, and transferred immediately to the fixative.

Rabbit No. 1.—Alternate sections of the brain and cord were put in:

1.—Lang's fluid	Mercuric chloride	5.
	Sodium chloride	6.
	Acetic acid	5.
	Water	100.

2.—Van Gehuchten's fluid { Acid acetic gl..... 10. Chloroform..... 30. Alcohol abs..... 60.

In each of these fluids the material was allowed to remain 12 hours. That subjected to the Lang fixation was then passed through graded alcohols 40%, 60%, 80%, to absolute. To alcohols 80% and absolute, tincture of iodine was added until decolorization ceased. Van Gehuchten's fluid was followed by absolute alcohol, changed frequently until all trace of acid reaction had disappeared.

Rabbit No. 2.—The central nervous system was fixed in situ, by injection through the ascending aorta of normal salt solution at 100° F., followed by a 10% aqueous solution of formalin at the same temperature. Brain, cord, and ganglia were then removed and placed in formalin 10%, followed by graded alcohols.

Rabbit No. 3.—Fixation of alternate slices in: 1. Absolute alcohol. 2. Formalin 5% for 13 hours, followed by graded alcohols.

Rabbit No. 4.—Van Gehuchten's fluid.

Rabbit No. 5.—Fixation of alternate slices in: 1. Van Gehuchten's fluid. 2. Formalin 5%.

Rabbit No. 6.—Fixation of alternate slices in: 1. Absolute alcohol. 2. Formalin 10%.

Embedding.—For most of the work celloidin was used. It allows of sections quite thin enough for comparative purposes. Sections 6 μ in thickness are easily obtained and may be taken in series so as to include the whole of a particular cell. With care in embedding, sections 4 μ thick may be made, and by painting a thin layer of celloidin over the block before each section it is not difficult to cut sections of 2 μ. Such thin sections are used only in studying the finer details of cell structure. A few segments were embedded in paraffine, which allows of even thinner sections and is a little more convenient for serial work. For general work I prefer the celloidin embedding. Unless otherwise stated, 6 μ is the standard thickness of section.

Section Cutting.—In addition to what has been said, I desire to emphasize the extreme importance of uniformity in thickness of sections. When one is dealing with such structures as those which make up the nerve cell, very different pictures are presented with varying thickness of section. It is therefore necessary in all comparative work, especially where the comparison is between normal cells and cells presumably pathological, to know the thickness of section and to compare only cells from sections of like thickness.

Staining.—For the greater part of the work the staining method used

is that of Nissl with modifications suggested by Held (6 and 20). Sections are first stained in a solution consisting of:

Erythrosin,	1
Aqua dest.,	150.

In this solution the sections are warmed for about 2 minutes, after which they are thoroughly washed in water and transferred to the following:

Methylene blue,	3.75
Venetian soap,	1.75
Aqua dest.,	1000.

To this is added after perfect solution an equal volume of a 5% aq. sol. of acetone. In this stain the section is heated until the acetone is driven off. It is then decolorized in a 0.1% aq. sol. of alum, washed in water, passed through alcohols and cleared in oil of cajeput. I have been accustomed to passing the specimens through alcohol after the erythrosin staining and washing with water, and think that a sharper picture is obtained by so doing. The specimen is then re-transferred to water before staining with methylene blue. I also prefer warm water for washing the specimens after both stains as there is less tendency to shrinkage of the specimen than when it is transferred from the hot staining fluid to cold water.

Of these rabbits, numbered 1, 2, 3, 4, 5, and 6, the following cells have been studied:

1. Stichochromes of the ventral horns and of the medullary nuclei.
2. Cells of the spinal ganglia.
3. Purkinje cells.
4. Mitral cells of the olfactory lobe.
5. Cells of the cerebral cortex.
6. Cells of the basal ganglia.

I. SPINAL AND MEDULLARY STICHOCHROMES.

Plate XXXIV, Figs. 1, 2 and 3.

In the cord these cells are found mainly in the anterior horn. Some occur in the central and posterior portions of the grey matter, and well out in the white matter along the course of nerve fibres. They are most numerous in the cervical and lumbar enlargements, and are arranged in what appear on cross sections to be ill defined

groups of cells, but which on longitudinal sections prove to be longer or shorter columns of cells. These groupings or columns are less distinct than in the human cord, and are not constant for different cords, for example, a section through the centre of exit of the second pair of lumbar nerves in one cord would not necessarily show the same groupings as a section through the corresponding locality of another cord. In the dorsal cord the cells are much fewer, and show but little grouping. In the medulla, these cells are found mainly in the formatio reticularis, which represents the breaking up of the ventral horn, and in the nuclei of origin of the cranial nerves. They vary greatly in size and shape, averaging 40 to 50μ, being rather smaller than similar cells of the human cord.

A. The Nucleus and Nuclear Contents.—The nucleus lies approximately in the centre of the cell. It varies in diameter from 15 to 30μ, averaging about 20μ; in general the larger cells have the larger nuclei, but there is less variation in size of nuclei than in size of cells.

The *nucleolus* is spherical, and is at the centre of the nucleus, or part way between the centre and the periphery. It varies in size from 2 to 6μ. It stains intensely with the basic anilines, and when so stained may appear perfectly homogeneous (Plate XXXIV, Fig. 1), or may show one or several round or oval areas of lighter color, resembling vacuoles. While reacting more intensely to basic dyes, the nucleolus is far from unstainable with acid dyes such as eosin and erythrosin, which stain it bright red. Rehm (25) counterstaining with a solution of carmine 1 part, liq. ammon. caustic 1 part, in water 100 parts for five minutes, then decolorizing five minutes in alcohol 100 parts, potassium nitrate 1 part, stained the centre of the nucleolus red, while leaving a peripheral rim of blue. Ewing (26), in sublimate-hardened specimens treated with Ehrlich's triacid mixture, obtained the same result, the conclusion being that micro-chemically the nucleolus is composed of two parts, a central acidophilic, and an outer basophilic zone. Rehm also claims this reaction for only one intranuclear body, which he considers the true nucleolus, the so-called secondary nucleoli found in normal nuclei, and the fragments fre-

quently present in pathological nuclei, reacting to only the single dye.

I have been unable to confirm these claims of a double microchemical staining reaction for the nucleolus. The exact appearance of the nucleolus is dependent, to a large extent, upon the method of staining and the details of its application. As has been stated, when stained with methylene blue alone, the nucleolus takes a homogeneous or vacuolated dark blue stain; the same is true when a light erythrosin stain is followed by strong staining with methylene blue. The erythrosin stain can, however, be made so strong, that when it is followed by a weak or moderately strong methylene blue stain, the red color remains. Upon the border line lie those nucleoli in which an overstaining with erythrosin, or an understaining with methylene blue allows the central portion to retain its red color, while covering over the surface with the blue stain. This gives the appearance of a blue rim with a red or purplish red centre. By continuing the alcohol decolorization of such a specimen the blue rim may be removed, leaving the entire nucleolus stained red. In some of my specimens fixed in formalin and in van Gehuchten's fluid, sections mordanted in 5% aqueous solution of copper bichromate, and then stained by the Weigert-Pall method with subsequent rather under-decolorization, a quite different appearance was presented by some of the nucleoli. In a homogeneous nucleolar ground substance, clear or faintly yellow, are scattered fairly evenly a number of small, irregular, dark brown, or black granules. By a $\frac{1}{18}$ immersion lens extremely fine faint lines are seen to extend off from these granules, giving the impression of a stainable intra-nucleolar reticulum traversing a clear unstainable ground substance. That such a structure could exist, and yet be entirely undemonstrable by such intense dyes as erythrosin and methylene blue is certainly within the range of possibility. The nucleolus showed no variation in appearance due to differences in fixation.

The intra-nuclear network or nucleo-reticulum.—Eliminating the nucleolus, two nuclear substances remain, the one stainable the other non-stainable. The former reacts rather faintly to methylene blue, strongly to erythrosin. It consists of a reticulum, and of coarse and

fine granules. These granules, especially the coarser ones, tend to collect around the nucleolus and to extend out upon the strands of the network, thus surrounding the nucleolus with a somewhat stellate mass. There is no question as to the acidophile character of this reticulum and of the granules, for, although they can be stained, the reticulum faintly, the granules quite strongly with methylene blue alone, when both stains are used they invariably choose the acid dye. The meshes of the reticulum are irregular and vary in diameter, being smaller near the centre and larger near the periphery of the nucleus, where the reticulum becomes continuous with the limiting membrane. This membrane appears from its staining reaction to be of the same composition as the intra-nuclear network. On further analysis this reticulum and membrane are seen to be covered, at least throughout the greater part of their extent, by fine acidophile granules. Whether there is underlying these granules a true fibrillar reticulum, or whether the whole apparent reticulum is not merely the result of a stringing together of granules, many of them beyond the power of the microscope to reveal, I have been unable to determine.

The remaining ground-substance of the nucleus shows no evidence of any structure, and with the erythrosin-methylene-blue stain gives no reaction. In specimens which have been treated with a less selective dye than erythrosin, e. g. with a strong alcoholic solution of ordinary eosin, the nucleus often takes a rather diffuse stain. This may be due to an actual staining of the ground substance or possibly to a retention of the dye within the fine meshes of the reticulum.

Under the different methods of fixation used, the nucleus and nuclear contents presented a very uniform appearance. Formalin 5% and 10% and van Gehuchten's fluid brought out most clearly the intra-nuclear network. It was especially clear in rabbit No. 2, in which the nervous system was fixed in situ with formalin 10%.

B. The Cell Body.—(*a*). *The stainable substance of Nissl* is present in the form of irregular masses or clumps of granules. These masses are of various sizes and shapes, are rather coarsely granular and arranged in a manner generally parallel to the outlines of the

cell body and of the nucleus (Plate XXXIV, Fig. 1). Around the periphery of the nucleus they are apt to form a fairly complete ring of blue granules, thus emphasizing the nuclear contour. Near the centre of the cell these so-called Nissl bodies are densely packed together, thinning out somewhat towards the periphery where there is often an area entirely or partly free from this substance. This tendency to central concentration and a peripheral clear zone, I have found more marked in formalin-hardened specimens, especially when a 10% solution was used, as in rabbit No. 2. As a protoplasmic process is approached the masses become more spindle shaped, until in the process itself the granules are strung out in long narrow rods parallel to the long axis of the process (Plate XXXIV, Figs. 1 and 2, a). The amount of this stainable substance is here much less, in proportion to the non-stainable, than in the cell body proper. That part of the cell in immediate proximity to the axone is free from blue staining granules.

In stained sections cut 6μ and over in thickness, the Nissl bodies are dense blue, often quite homogeneous in appearance at their centres, and shading off to a lighter blue at the edges which always look granular. The thinner the section, the less extensive is the dense central portion and the larger is the peripheral granular area, and in sections of 2μ and under the entire mass is granular. In such sections and even in much thicker sections it is evident that the granules which make up one of these masses are not evenly distributed, being thickly packed in some places and in others farther apart. This gives sometimes a vacuolated appearance to a mass. Reference has been made (p. 554) to Held's description of a distinct embedding mass or coagulum in which the granules lie, this coagulum staining a violet color in contra-distinction to the blue of the basophile granules on the one hand and to the red of the acidophile element on the other. In none of the rabbit's nerve cells which I have studied, either normal or pathological, have I been able to make out any distinct embedding mass in which the granules are placed. That such a mass may exist is not at all improbable. When we consider that in the contents of nerve cells we are dealing with molecules of the highest complexity,

and hence of the greatest instability as well as potentiality, it would be rather surprising if there were not in these Nissl bodies, whatever their relation to the living cell, various constituents differing from one another in both chemical and physical features. In specimens stained with erythrosin alone the Nissl bodies take a red stain; when placed in the methylene blue solution, their greater affinity for that dye, or its greater intensity causes them to change to a blue color. My observations, however, have been that there is in this process a certain covering-over of one color by the other, and not an entire replacement of the red by blue; for, in most cases, if, after the staining with methylene blue, the decolorization be sufficiently pushed (especially if the specimen has been washed in alcohol after the erythrosin staining and water washing), the blue may be entirely removed and the Nissl bodies will again be seen stained pink or red. Again it is noticeable that at the surface of a chromophilic body, where the individual granules are separated from one another and more distinct, there is no appearance of a substratum of violet color, and that the more compact the mass of granules, the more clearly is the violet color seen. Furthermore, the shade, toward blue or toward red, can be varied at will by regulating the intensity of the methylene blue stain and the subsequent decolorization. It would thus seem that, while an embedding mass for the chromophilic granules, of different composition and staining reaction than the granules themselves, may exist, the appearances observed in these rabbit's cells can be explained on purely physical grounds.

In specimens stained with erythrosin and methylene blue, two constituents of the cell body remain after elimination of the chromophilic bodies: one of these is stained by erythrosin and hence denominated acidophile, the other remaining unstained we will speak of as the truly achromatic element of the cell.

(*b*). *The acidophile constituent* shows most clearly in specimens stained with erythrosin and methylene blue after fixation in formalin or van Gehuchten's fluid. It takes the form of fine anastomosing threads, giving the appearance of a distinct reticulum. In the parts of the cell where the basophilic element is most abundant, the ap-

pearance of the reticulum is largely obscured. It is seen to best advantage near the periphery of the cell and in the larger protoplasmic processes (Plate XXXIV, Fig. 2). Comparing it with Ewing's description and drawing of the reticulum found in the large stichochromes of the human ventral horn, the threads of this reticulum (rabbits' anterior horn cells) appear finer, the nodal points rather more marked. The size of the mesh is fairly uniform, averaging from 1 to 3 μ in diameter. The shape of the mesh is irregular and the strands of the reticulum are sometimes quite smooth and again wavy, looking very much like the outline of omental endothelium as seen in silver-nitrate preparations. As the reticulum approaches a protoplasmic process, its meshes elongate, and in the processes themselves we have a reticulum with long, narrow irregularly rectangular meshes (Plate XXXIV, Fig. 2, a). This reticulum extends out into very fine dendritic branches, in fact I have failed to find any dendrites, however small, in which there was no trace whatever of the acidophile reticulum, though it was sometimes reduced to a single fibril. As the reticulum approaches the axis cylinder process, on the other hand, the appearance of an anastomosing network is gradually lost, by the disappearance of the connecting fibrils, the reticulum passing over into, and being continuous with, fine parallel fibrillæ which lie close together and compose the axis cylinder (Plate XXXIV, Fig. 2, c).

As to the ultimate structure of this reticulum, I am inclined to accept Held's idea already referred to, that it is made up of rows of staining granules upon the limits of microscopic perceptibility. With a Reichert $\frac{1}{18}$ immersion lens the appearance of the reticulum was not always altogether uniform. Some parts of the reticulum could be resolved into extremely fine but distinct granules; others looked like entirely homogeneous, anastomosing fibrils. The fact, however, that portions of the reticulum appear granular in structure, these granules being at the limit of microscopic vision with the highest powers, favors the hypothesis of a granular structure of the entire reticulum, many of the granules being so fine as to be unrecognizable as such.

The relation of the basophile granules to the acidophile reticulum

is seen to best advantage in extremely thin sections after fixation in formalin or van Gehuchten's fluid and staining with erythrosin and methylene blue (Plate XXXIV, Figs. 2 and 3). It shows most clearly in those parts of the cell where the basophile element is present in small amounts, e. g., at the periphery of the cell and especially in some of the longitudinal sections of the protoplasmic processes. Where the Nissl bodies are larger and more numerous, they extend over so many meshes of the reticulum and overlap each other in such a way as to obscure largely the acidophile network. The basophile granules seem to be laid down upon and around the strands of the acidophile reticulum, forming, as it were, a sort of incrustation (Plate XXXIV, Fig. 3). Each of the larger blue masses covers over several meshes of the reticulum and only at their edges can the fine rows of granules extending off into the delicate lines of the reticulum be seen. In extremely thin sections fine pink lines and irregular red spots (Plate XXXIV, Fig. 2, b) may sometimes be seen within the Nissl bodies, especially near their edges, which would seem to be strands and nodal points of the acidophile reticulum. In transverse sections of protoplasmic processes the long chromatic rods are seen in cross section and appear round, oval or somewhat irregular. These often show a minute pink dot (Plate XXXIV, Fig. 2, b), usually situated near the centre of the granule. This dot is sometimes so minute as to be barely visible; sometimes larger and more irregular. There may be several pink points instead of one. I believe that these pink dots represent strands of the acidophile reticulum in cross section and that the larger and more irregular bits of pink represent sections through nodal points of the reticulum.

(*c*). There remains that constituent of the cell which with the erythrosin-methylene-blue staining is entirely *achromatic*. The description already given of the achromatic part of the nucleus (p. 561) applies equally to the achromatic part of the cell body. It is unstained and apparently structureless. Certain other dyes, however, either stain this ground substance or are retained within the meshes of the reticulum.

I have repeated with some modifications the experiments of Held (6) in examining fresh nerve cells, with the following results:

The specimens consisted of bits of the ventral horns from the lumbar cords of rabbits and guinea-pigs. The animals were chloroformed, the bits of cord removed as soon as anæsthesia began, and quickly teased apart in the following media, all at a temperature of 100° F.:

1. Solution of sodium chloride 0.6%. Nucleus and nucleolus distinctly visible; no evidence of any chromophilic bodies.

2. 0.6% sol. sodium chloride, to which was added sodium hydrate to 0.224%, or about the alkalinity of the blood. No chromophilic bodies seen.

3. 0.6% sol. sodium chloride, to which was added sufficient hydrochloric acid to have neutralized, volume for volume, the above alkaline solution. In this preparation indistinct irregular clumps of granules were visible which stained on the addition of methylene blue.

4. 0.6% sol. sodium chloride, to which was added methylene blue to 0.01%. No chromophilic bodies could be seen.

5. 0.6% sol. sodium chloride, plus sodium hydrate to 0.224% plus methylene blue to 0.01%. Result negative.

6. 0.6% sol. sodium chloride, plus hydrochloric acid sufficient to have neutralized an equal quantity of the above alkaline solution, plus methylene blue to 0.01%. This preparation showed distinct and well formed chromophilic bodies.

7. 0.6% sol. sodium chloride plus 0.1% methylene blue. Showed distinct chromophilic bodies.

8. 0.6% sol. sodium chloride, plus sodium hydrate to 0.221%, plus 0.1% methylene blue. Showed no chromophilic bodies.

9. 0.6% sol. sodium chloride, plus 1% methylene blue. Distinct chromophilic bodies.

The results may be summed up as follows:—

(1). Nerve cells examined immediately after removal in warm normal saline solution neutral in reaction, in normal saline solution of approximately the alkalinity of the blood, and in the same plus a small amount of methylene blue (0.01%), showed nothing corresponding to Nissl bodies.

(2). In warm normal saline of slightly acid reaction, an indistinct clumping of granules was observed, which stained on the addition of methylene blue and resembled chromophilic bodies.

(3). In normal saline of slightly acid reaction plus methylene blue (0.1% or 0.01%), and in normal saline of neutral reaction plus methylene blue (0.1% or 1%), distinct chromophilic bodies were found.

Such observations tend to confirm the belief expressed by Held that the chromophilic bodies in the Nissl preparations represent merely the precipitation—the result either of post-mortem changes or of the action of fixatives—of a substance or substances previously held in suspension or in solution in the protoplasm of the living cell.

An attempt was also made to stain the cytoreticulum of the fresh cell with erythrosin, but with negative result. Such failure, however, does not disprove the existence of such a reticulum in the living cell, inasmuch as its structure is so delicate and requires such careful technique that it is difficult of demonstration even in hardened preparations. From observations upon both fresh and hardened tissues I am of the opinion that, whether existent in the living cell or not, whether a true reticulum or some such structure as that described by Bütschli, the strands of the acidophile mesh are formed before the chromophilic bodies, and that the latter are the result of a precipitation of fine granules which are caught in and upon the meshes of the reticulum.

2. NERVE CELLS OF THE SPINAL GANGLIA. *Plate XXXIV, Fig. 4.*

Ewing, in his "Studies on Ganglion Cells," sums up our knowledge of the human spinal ganglion cell, resulting from the investigations of von Lenhossék, Held, Flemming, Nissl, Cox, and Heimann, as follows: The cells vary from 60μ to 80μ in diameter, sometimes reaching 120μ. Each cell is enclosed in a connective-tissue capsule lined by a single layer of endothelium. In the normal living condition each cell completely fills the enclosed space. The cells are unipolar, the axone taking origin as usual from an achromatic area. Flemming describes three varieties according to the size and arrangement of the chromatic masses. Cox mentions two varieties—one in which small irregular chromatic masses are present without definite concentric arrangement, the other in which large irregular chromatic bodies are arranged concentrically. Ewing in human beings, and Heimann in the rabbit distinguish but a single type of spinal-ganglion cell, and with this my own observations coincide.

The cells of the spinal ganglia in rabbits (Plate XXXIV, Fig. 4)

vary in size from 20 to 80 μ. They are round or show sides flattened by pressure of contiguous cells. The nucleolus presents the same appearance as in the ventral horn cells. It is common for a nucleus to have two or more nucleoli. The intra-nuclear network is rather coarser than in the spinal stichochromes. The arrangement of the basophile substance varies so greatly as to have led some investigators to differentiate several types of ganglion cells. It always appears granular and the granules are as a rule quite coarse. They may be scattered fairly evenly throughout the cell with no arrangement into chromophile bodies (Plate XXXIV, Fig. 4, b), or there may be a peripheral zone or both peripheral and peri-nuclear zones, partly or entirely free from chromatic granules. In other cells the granules are collected into distinct masses, very irregular in shape, but having, in general, concavities towards the nucleus and convexities towards the periphery of the cell (Plate XXXIV, Fig. 4, a and a′). Taking the extremes of these cells, it is easy to construct a description of two distinct types of ganglion cells. Between these extremes, however, all gradations exist, and these, in view of the similarity in shape, the uniform unipolarity and the constant connective-tissue capsule, suggest minor variations, probably physiological, in a single type of cell, rather than several distinct types.

The cyto-reticulum in these cells is fine both as to its strands and size of mesh. It is usually clearly seen near the periphery of the cell but much obscured in the deeper parts. The relation of the basophile granules to the acidophile reticulum is very well demonstrated in some of these cells, and is the same as that described in the spinal stichochromes.

3. NERVE CELLS OF THE CEREBELLUM. *Plate XXXIV, Fig. 5.*

In the cerebellum, the only cells of special interest when subjected to the method of Nissl, are those of Purkinje. Formerly classed as arkyostichochromes, these cells Nissl now includes in the arkyochrome group. They lie as a distinct row of cells at the junction of the molecular layer with the granular layer, their cell bodies embedded in the latter. The point which makes them of most interest when

subjected to this method of staining, is their possible function as the motor cells of the cerebellum, presiding over the government of equilibrium. This and the fact that they seem to be rather quickly and easily affected by pathological conditions, adds interest to a close study of their normal appearances.

The nucleolus presents the same appearance as in the cells already described. It is not uncommon to find two, three, or even more nucleoli. The nucleus is small, in proportion to the size of the cell, round, and situated at the base of the cell. The intra-nuclear network may be either coarse or fine meshed.

The chromatic substance (Plate XXXIV, Fig. 5) is arranged somewhat like that in the spinal ganglion cells, i. e., masses of granules lying as longer or shorter curved rods with their long axes parallel to the contour of the nucleus and of the cell periphery, and their concavities directed centrally. The cells differ from those of the spinal ganglia in the wider separation of the Nissl bodies, and in the much closer packing of the extremely fine granules which make up a chromatic mass. These granules in many cases are so fine as to give the chromophilic bodies a homogeneous appearance. The chromatic substance is most abundant near the centre of the cell, becoming rapidly less towards the main dendrite. In most of these cell bodies, the cytoreticulum is indistinct, owing, often, to the diffuse staining of the ground substance. In the neck of the cell, and in the main dendrite, where the mesh becomes elongated, there is an extremely clear demonstration of the cytoreticulum and of its relation to the chromophilic bodies. There may be a well marked nuclear cap of chromophilic substance (Plate XXXIV, Fig. 5, b), and the chromatic pyramid at the bifurcation of the main protoplasmic trunk is quite large. Chromatic rods are found in the smaller dendrites as far as they can be traced in the section.

4. NERVE CELLS OF THE OLFACTORY LOBE. *Plate XXXV, Fig. 6.*

The accompanying diagrams, Figs. A and B, of the exterior of the rabbit's brain show the situations from which sections of the olfactory lobe and of the cerebral cortex were taken. The dimensions

are approximately normal for a medium-sized rabbit. The localization given is that of Mann. Speaking generally, this localization of function is probably fairly accurate, though most other experimenters have failed to make out quite as definite limits to the functional areas.

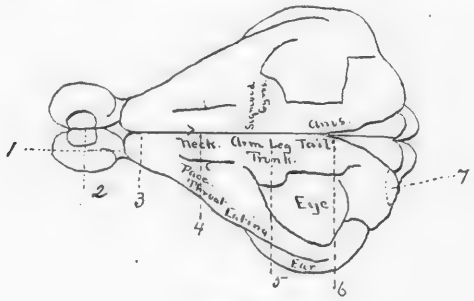

FIG. A.

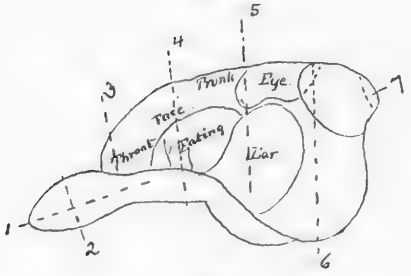

FIG. B.

FIGS. A and B.—Represent Gross Anatomy of Surface of Rabbit's Brain. Localization according to Mann.

FIG. A.—Rabbit's Brain. Dorsal view.

FIG. B.—Rabbit's Brain. Lateral view. Dotted lines show situations from which sections were taken.

The dotted lines of Fig. B represent points at which sections were taken. Owing to the very considerable difference in size of brain in large and small rabbits, these points were fixed by bisecting the distance between two fixed points, rather than by a definite measurement in millimetres from any one point.

Fig. C is a low power drawing, slightly diagrammatic, from an antero-posterior section through the middle of the olfactory lobe,

corresponding to dotted line 1, Fig. B, extending from the surface to the ventricle. The section was stained with methylene blue alone. It shows the layers of the olfactory lobe as given by Ramon y Cajal. Van Gehuchten examining these cells by Golgi's method interprets the appearances as follows:

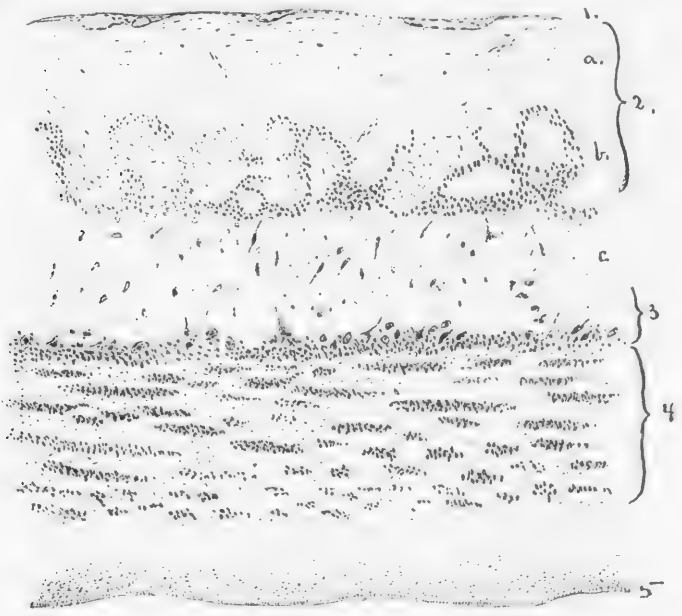

Fig. C.

Low Power Drawing, slightly diagrammatic, of Antero-posterior Section through Middle of Olfactory Lobe, corresponding to dotted line 1 of Fig. B, from surface to ventricle.

1. Pia Mater.
2. { *a.* Layer of Olfactory Fibres.
   { *b.* Layer of Olfactory Glomeruli.
   c. Inferior Molecular Layer.
3. Layer of Bipolar Cells (Mitral Cells).
4. Layer of Nerve Fibres and Granules: the Superior Molecular Layer.
5. Epithelial Layer.

1. Lying just underneath the pia mater is a layer formed by bundles of the peripheral olfactory nerve fibres. These are the axis-cylinder processes of the bipolar cells of the olfactory mucous mem-

brane. These axis-cylinder processes terminate freely, either directly or after bifurcation, in the glomerules.

2. Beneath this is a layer of grey matter rich in nerve cells. These are mostly of the bipolar type, and are called the layer of bipolar cells. At the junction of this layer with the next internal one, a considerable number of these cells are arranged in a row, much after the fashion of the Purkinje cells of the cerebellum. The axis cylinder processes of these bipolar or mitral cells pass downward into the white matter. Their protoplasmic processes terminate either freely or by arborization in the olfactory glomeruli.

3. An internal layer, formed by bundles of nerve fibres of the olfactory tract, intermingled with groups of small nerve cells.

From Fig. C and the description just given, it will be observed that the great mass of the cells of the olfactory lobe are of the "granule" type, corresponding in appearance to the cells of the granular layer of the cerebellum. These are caryochromes and consequently possess little interest when stained by the method of Nissl. The layer of mitral cells, however, is composed of somatochromes which react characteristically to the Nissl technique.

The section from which the cells in Plate XXXV, Fig. 6, are taken, is a median transverse section of the olfactory lobe corresponding to dotted line 2, Fig. B. Both cells are situated in the line of bipolar cells marking the outer border of the internal granular layer. Cell a, Fig. 6, shows two processes, the one to the right passing downward into the granular layer, the one to the left passing toward the periphery. Both of the processes of cell b pass in the latter direction.

1. The nucleolus is usually eccentrically situated. It does not differ from the nucleoli already described. There may be more than one.

2. The nucleus is large in proportion to the size of the cell, is spherical or slightly ovoid in shape—in the latter case its long axis corresponding to the long axis of the cell—and is usually situated in the centre, more rarely near the inner surface of the cell. The intranuclear network is rather coarse, is well defined, stains strongly, and there is a rather large amount of granular acidophile substance just around the nucleolus.

3. The chromophilic bodies are of all sizes and shapes. They are coarsely granular, the granules being placed very closely, thus giving an almost solid appearance to the bodies unless the section be extremely thin or the bodies viewed at their peripheries. There are always present several large masses of chromatic substance, larger than those found in any other nerve cell, excepting those of the cornu Ammonis and some of the spinal and medullary stichochromes; they tend to be triangular, and to group themselves near the centre of the cell, around the nucleus. The "nuclear cap" is almost always well marked and a row of finer granules accentuates the outline of the nucleus. Besides these larger masses which vary in number, numerous smaller bodies are scattered throughout the cell. In the form of larger or smaller rod-like masses, the chromophilic substance extends out into the protoplasmic processes, usually as far as the section allows the processes to be traced. The chromophilic bodies of these cells stain intensely with methylene blue, and the rest of the cell body, being decolorized with considerable difficulty, often retains a faint blue tint.

4. The cytoreticulum is obscured in the central part of the cell by the large closely packed chromophilic bodies. Around the edges of the cell, however, and in the dendrites, it is clearly seen.

5. NERVE CELLS OF THE CEREBRAL CORTEX.

Plate XXXV, Figs. 7, 8 and 9.

The layers of the rabbit's cortex cerebri, as given by van Gehuchten, correspond to those of the human cortex.

1. The superficial layer, which has been called the barren layer or, more properly, the layer of few nerve cells, is made up almost entirely of non-medullated nerve fibres, which pass in a direction tangential to the surface. Many of these fibres are axis-cylinder processes of cells of the deeper layers; others are association fibres, being the cortical terminations of cells from other convolutions.

Scattered among these fibres are nerve cells, of which van Gehuchten recognizes three types: (a) A polygonal cell having four or five large protoplasmic processes, which pass in all directions, and an

axone, which, originating from the lateral aspect of the cell, runs parallel to the surface and terminates in this same layer. (b) A fusiform cell which lies horizontal to the surface, its long axis running antero-posteriorly. It has but these two dendrites which are so long that they are rarely wholly included in even a thick section. This cell usually has more than one axis-cylinder, and these spring from the dendrites at some distance from the cell body. The processes of this cell also terminate in this same layer. (c) A triangular cell with two dendrites passing laterally and a third passing downwards; there are several axones which spring from the dendrites.

2. Beneath the barren layer is one of small pyramidal cells. The axones of these cells pass downward to become medullated nerve fibres either of association or of radiation.

3. Without any distinct line of demarcation from the preceding, is a layer containing a considerable number of much larger cells of the same shape; this is the layer of large pyramidal cells. Their axones take the same course as those of the small pyramidal cells.

4. The layer of polymorphous cells. The axones of these cells pass, some downward, like those of the pyramidal cells, others upward to terminate in the cortex.

The regions of the cortex from which sections have been taken for study are indicated by the dotted lines in Fig. B (p. 570).

1. At line 3, about 2 millimetres back from tip of frontal lobe. Plate XXXV, Fig. 7.

The cells are mostly small and without any great variation in size between the layer of small pyramidal cells and that of large pyramidal cells.

The nucleolus is commonly eccentric and presents the same appearance as already described. Several nucleoli are often found. The nucleus is round or ovoid, rarely slightly irregular in shape. It is situated either at the centre of the cell, or in pyramidal cells near the base. The intra-nuclear network is well defined and rather coarse and stains dark red. There is apt to be a considerable amount of granular acidophile matter around the nucleolus.

The basophile constituent of the cell body varies greatly as to

amount and distribution in different cells. In most of the small pyramidal cells the collections of basophile granules are small, irregular in shape, and scattered very unevenly throughout the cell body. Plate XXXV, Fig. 7, b, shows a vertical section through such a cell, while Fig. 7, a, represents a section at right angles to the long axis. Most commonly one or two small chromatic masses are found, the remainder of the chromatic substance being in the form of fine separate granules which, lying on the strands of the acidophile reticulum, give the appearance of a blue network (Plate XXXV, Fig. 7, b and c). In most of the large pyramidal cells this same condition obtains. In some of the larger cells, however, are found Nissl bodies of more definite size and shape (Plate XXXV, Fig. 7, b). A large part of the cell body is often clear of chromatic substance. Rarely such a cell as Fig. 7, d, is found, in which a rather small nucleus is situated near the base of the cell and chromophilic bodies of fairly large and uniform size are distributed rather evenly throughout the cell.

The acidophile reticulum is extremely delicate, the mesh being small and the strands fine; otherwise it is identical with that found in other cells. The proportionately small amount of basophilic substance often allows the reticulum to stand out very sharply (Plate XXXV, Fig. 7, c).

The finer chromophilic bodies stain with methylene blue very lightly and are decolorized quickly. Even the larger chromatic masses of these cells show a disinclination to retain the dye, and, if the sections remain long in the aniline-oil alcohol, or even in strong alcohol, they are more or less completely decolorized.

2. At line 4 (Fig. B), equidistant from tip of frontal lobe and the line of mid-section.

The general arrangement of the cells here is the same as that just described. The cells of the small pyramidal layer are of about the same size as in the same layer of the preceding section. In the layer of large pyramidal cells, however, are many cells much larger than any found in the corresponding layer of the frontal lobe.

As regards the nucleolus, nucleus, and intranuclear network, the foregoing description applies. The nucleus varies greatly in size, bearing a fairly definite relation to the size of the cell.

The chromophilic bodies in these cells appear much the same as those shown in Plate XXXV, Fig. 7. The preponderance, however, of cells in which the chromophilic bodies are little more than granules, is no longer so marked. There are still, however, more of these cells than of any other type, especially in the superficial layer. Further down in the layer of large pyramidal cells the average size of the cells is much greater than in the corresponding layer of the frontal lobe, and there is a much larger number of cells fairly well filled with chromophilic bodies of considerable size and distinctness. Many of the larger cells, however, present but little chromatic substance in proportion to the size of the cell body. These large cells tend to arrange themselves in ill defined groups of from three or four to a dozen or more.

3. At line 5 (Fig. B). Transverse median section taken at an equal distance from the tip of the frontal lobe and the tip of the occipital lobe. Plate XXXV, Fig. 8.

The cells in this region of the cortex correspond somewhat to those already described. There are the same different types of cells as regards their chromophilic bodies, but a still further change in the numerical relation between them, and the cortical layers are better demarcated. The grouping of the large cells is more pronounced and the groups are larger than in the preceding sections. The cells are larger and their number proportionately much greater. In these large cells there may be a very small amount of basophile substance as in Plate XXXV, Fig. 8, a, and then the acidophile reticulum is distinct. In other large cells there is a considerable amount of chromatic substance and the granules are collected into quite large masses (Plate XXXV, Fig. 8, b and c). There are often very distinct nuclear caps.

4. At line 6 (Fig. B), equidistant from the tip of the occipital lobe and the mid-point.

There is again a preponderance, although not quite so marked as in the frontal lobe, of small cells, having very fine and indistinct chromophilic granules. In the layer of large pyramids there are fewer large cells, and these are scattered and show less tendency to grouping. Otherwise the description already given applies.

5. At line 7 (Fig. B). The tip of the lobe was removed and sections were then made through the tip of the prominence at right angles to a line tangential to the surface at that point. Plate XXXV, Fig. 9.

As we pass from the region last described, we find that there is here a decided diminution in the number of cells of the larger type. All of the cortex from the barren layer to the layer of large cells is occupied by extremely small cells. These cells have a small nucleus and a small cell body, sometimes a few fairly well defined chromophilic bodies, more often only a few fine granules.

The large pyramidal cells are much smaller than in the central regions of the brain, and are scattered irregularly and rather sparsely throughout the layer. Otherwise they resemble the large pyramidal cells of other parts of the cortex. Of the cells pictured in Plate XXXV, Fig. 9, those of type d greatly predominate in this region; c and b are found in moderate numbers, and type a is rare.

The results of this study of the rabbit's cortical nerve cells (Figs. 7, 8, and 9) indicate that certain types may be distinguished as follows:

1. The large pyramidal cell. This is found in all regions of the cortex, but the cells are much more numerous and of much larger size in the mid-region of the brain, that is about the region analogous to the fissure of Rolando in the human cortex—the general sensory-motor area. In this region these cells are in more or less definite groups. As we pass either anteriorly or posteriorly these large pyramidal cells decrease in both size and number, until in the frontal and in the occipital lobes they reach their minimum of size and frequency. These cells, in sections treated by Nissl's method, present three fairly distinct types, as regards the arrangement of their chromophilic bodies, these types, however, merging into one another without distinct dividing lines.

(a). The large pyramidal cell in which the chromophilic bodies are small and few, the greater part of the cell appearing clear (Plate XXXV, Fig. 8, a). This is the predominating type of large pyramidal cells in all regions.

(b). The large pyramidal cell in which there is more chromatic substance in proportion to the size of the cell than in type (a) (Plate XXXV, Fig. 8, c, and Fig. 9, a). The chromophilic bodies are of considerable size, irregular in shape, and are largest in the region around the nucleus, where they form distinct nuclear caps. In these cells the chromophilic bodies, though quite numerous and of fair size, by no means fill up the cell, there being a fair amount of clear cytoplasm. They are distributed throughout the entire cortex but are more numerous in the mid-region.

(c). The large pyramidal cell in which the chromophilic bodies are quite large, and of fairly uniform triangular shape and size (Plate XXXV, Fig. 7, d, and Fig. 8, b). They are scattered rather densely and evenly throughout the cell body, giving the cell much the appearance of a motor ganglion cell of the spinal cord. These are the least frequent of all the cells of the cortex.

2. The small pyramidal cell. This is the predominating cell of the cortex. The arrangement of its chromatic substance presents two types.

(a). The small pyramidal cell in which there are only a few fine chromophilic granules scattered irregularly throughout the cell body, most of the cell remaining clear (Plate XXXV, Fig. 7, c, and Fig. 9, b, and d). These correspond to type (a) of the larger pyramidal cells. They are the most numerous cells of the cortex.

(b). The small pyramidal cell, containing several chromophilic bodies of fair size, corresponding in general arrangement to type (b) of the larger pyramidal cells (Plate XXXV, Fig. 7, b, and Fig. 9, c).

3. Irregular cells scattered throughout all parts of the cortex, but most numerous and largest in the deeper layers, and in the mid-region of the brain. They vary greatly as to size and arrangement of their chromatic elements, from a few scattered granules to large regular chromophilic bodies resembling those found in the spinal stichochromes.

6. NERVE CELLS OF THE BASAL GANGLIA.

Nucleus Caudatus.—The majority of the cells of this nucleus are small multipolar elements possessing no characteristics distinguishing

them from similar cells found in other parts of the nervous system. They vary in diameter from 10μ to 20μ. The nuclei are round or slightly oval in shape, with, as a rule, a single centrally placed nucleolus. In the larger of these cells the nucleus is surrounded by a considerable area of cell body, in the smaller the cell body is reduced to a mere rim surrounding the nucleus. The acidophile reticulum is extremely fine-meshed but presents the same appearance as in cells already described. The amount of the basophile substance present is extremely variable, as is also its distribution within the cell body. In most of the cells the amount is rather small and the granules lie upon the strands of the reticulum. In cells where more of the basophile element is present it is apt to accumulate along the outside of the nuclear membrane, at the periphery of the cell, and as irregular clumps of granules near the origins of the main dendritic processes.

Among these smaller cells are scattered a few much larger ones corresponding in type to the spinal stichochromes. They seem to differ in no way from the smaller ventral horn cells.

There is also a considerable number of cells in which the basophile substance is present as fine granules giving a blue dusty appearance to the cell body. These cells Nissl describes as "gryochromes."

Nucleus Lenticularis.—The cellular elements of this nucleus resemble those just described in the nucleus caudatus. In the sections which I have examined the small nerve cells average rather larger and the large stichochromes are larger and somewhat more numerous than in the caudate nucleus. In both nuclei the closeness with which the small cells are packed together and the number of large cells which are scattered among them vary greatly in different parts.

Optic Thalamus.—This region of the basal ganglia presents an extremely complex arrangement of its nuclei. The differentiation of the thalamic nuclei of the rabbit has been carefully worked out by Nissl, who distinguishes:

1. An Anterior Nucleus. This is situated in the most anterior part of the thalamus, and is separable into a larger part—the anterior ventral nucleus, and a smaller part—the anterior dorsal nucleus. In

the lower and outer portion of the anterior ventral nucleus, the cells have a quite compact arrangement and allow the differentiation of a lateral ventral, from a median dorsal area.

2. A Medial Nucleus. This nucleus lies near the median line, in much the same position as that occupied more anteriorly by the anterior nucleus. From the main body may be separated off a small anterior segment or anterior medial nucleus, leaving a larger remaining nucleus lying near the median line and extending about one-half the length of the thalamus.

3. Nuclei of the Reticular Zone. These are divisable into an upper dorsal part lying just below the anterior ventral nucleus, and a lower ventral portion. To the outer side of the latter is a small nucleus, the lateral nucleus of the reticular zone.

4. A Nucleus of the Middle Line. This is a flat plate of cells lying near the middle line. The projection inward of the main portion of the medial nucleus divides this nucleus throughout the greater portion of its extent into a dorsal and a ventral part.

5. A Lateral Nucleus. This is divided into a larger anterior part, extending through about two-thirds the length of the thalamus, and a smaller posterior part.

6. A Ventral Nucleus. This contains three main groups of cells, designated according to their location as the dorsal, the medial, and the lateral divisions of the ventral nucleus.

7. A nucleus lying close to the tænia thalami.

My studies of sections through the thalamic regions have been entirely confirmatory of those of Nissl. The main nuclear masses are easily distinguished, especially if followed from section to section. Isolated sections are sometimes confusing owing to the fact that at some point or points nuclei run into one another without any distinct demarcation. This is true especially of the posterior lateral nucleus, which at various points is continuous with the neighboring nuclei; for the same reason, there is sometimes difficulty in differentiating the subdivisions of the main nuclei. Again, neither shape, size, nor position of the various nuclei is absolutely fixed, there being some variation, usually slight for different rabbits.

No new types of nerve cells present themselves in these nuclei. The majority of the cells are of the small multipolar variety, resembling those found in the caudate and lenticular nuclei. There is not, however, that diffuse scattering of large stichochromes found in the nuclei of the corpus striatum. These large cells of the type of the anterior horn cells, are found mainly in the postero-lateral nucleus, which is made up mostly of large cells, the largest found in the thalamus. The median ventral nucleus also contains comparatively large cells. In the nucleus of the middle line and in the lateral ventral nucleus the cells tend to the fusiform variety. As is common in this form of cell, the basophile element is present mainly at the ends of the nucleus directed towards the main dendrites.

II.

CHANGES IN THE NERVE CELLS IN RABIES PRODUCED BY SUBDURAL INOCULATION OF RABBITS WITH FIXED VIRUS.

PLATES XXXVI–XXXVIII.

HISTORICAL REVIEW.

Although it is not the purpose of this article to consider the pathology of rabies further than the presentation of personal observations, mostly relating to changes in the nerve cells of rabbits inoculated beneath the dura with fixed virus, it will not be out of place to present a brief review of our knowledge of the pathological anatomy of this disease.

Observations upon the post-mortem appearances in rabies date back to the essay of Richard Mead (32) in the beginning of the eighteenth century and to the writings of van Swieten (33) later in the same century. They found no lesions in any way characteristic of the disease. Nor in the many recorded autopsies since these writers have characteristic gross lesions been detected. Congestion, œdema and small hæmorrhages in various parts of the central nervous system and degenerations such as are common to many infections have been noted. The claims of Gamaleia (34) and Schaffer (35) that macroscopic areas

of softening and necrosis in the spinal cord are characteristic of rabies have not been substantiated.

Even before the important discoveries of Pasteur, and more positively since these, it has been clear that the essential seat of rabies is in the central nervous system, and consequently there have been many careful microscopic studies of this part in recent years. These have brought to light the existence of lesions, mostly of a focal or miliary character, connected partly with the blood-vessels and partly with the nerve cells, the former being the ones chiefly emphasized until quite recently. Whether or not these lesions be regarded as in part characteristic, it has been established that rabies is no longer to be ranked among diseases without a definite and demonstrable anatomical basis.

The most important of the vascular lesions, as was pointed out a quarter of a century ago by Benedikt (36), Kolessnikow (37), Coats (38) and Gowers (39), is an accumulation of leucocytes around the blood-vessels, particularly in the spinal cord, medulla oblongata, and basal ganglia. Other vascular lesions frequently found are congestion, irregular dilatations, thrombosis, hæmorrhages, especially within the perivascular lymph-spaces, the deposit of hyaline both within and around vessels, and swelling of the endothelial cells.

The accumulation of leucocytes is not limited to the immediate neighborhood of the blood-vessels. They may appear around the central canal and in the nervous tissue, and especial importance attaches to their accumulation around nerve cells, particularly the motor ganglion cells, as pointed out by Babes (40) and Schaffer (35). To these pericellular and perivascular focal accumulations of small round cells, more especially to the former, Babes has given the name of "rabic tubercles" or nodules, and he considers that these are so characteristic of rabies that, when due attention is given to their intensity and distribution, they can serve as a diagnostic criterion of the disease.

In addition to these exudative hæmorrhagic lesions referable to the blood-vessels, various changes, mainly of a degenerative nature, have been found in the nerve cells both of the cerebro-spinal axis and of the ganglia. Schaffer described atrophy, vacuolation, and granular, hyaline and fibrinous degenerations of nerve cells associated with degeneration of the processes and hyperplasia of the neuroglia. Confirmatory and additional observations have been mabe by Babes, Gianturco (41), Golgi (42), Germano and Capobianco (43), and others. Thus Golgi, working with his silver method, has described irritative and regressive changes in the nucleus, characterized by increase and subsequent fragmentation of the chromatin with karyolysis, bladder-like swelling, vacuolation

and granular degeneration of the cell-body, atrophy and loss of the processes, and eventually destruction of the entire cell. The most extensive alterations, terminating in complete disappearance of nerve cells, were noted by Germano and Capobianco. Only certain cells were affected, others in varying numbers and distribution remained normal.

It is to be emphasized that the lesions described were observed, at least in a marked degree, mainly in street rabies. Babes says that in rabbits inoculated with fixed virus there is usually no lesion of the nerve cells—a conclusion not in accord with my observations.

The lesions described have been usually interpreted as manifestations of a disseminated exudative and parenchymatous encephalomyelitis.

Such in brief outline was the general state of our knowledge of this subject when there appeared early in 1900 the notable papers of van Gehuchten and his pupil, Nelis (44), which have stimulated other contributions. This later literature has been the subject of an admirable critical review by Crocq (45). These recent studies have drawn attention to an almost forgotten observation of Pollaillon and Nepveu (46), who in 1872 described in a case of human rabies the accumulation of round and oval cells around degenerated nerve-cells in the Gasserian ganglion.

Van Gehuchten and Nelis contend that the vascular and cellular changes described by previous investigators, including the "rabic tubercle" of Babes, are inconstant and secondary, not characteristic of rabies and unimportant for diagnosis of the disease, whereas the rabic pericellular nodules in the cerebro-spinal and sympathetic ganglia are so pathognomonic as to constitute a valuable means of rapid diagnosis of rabies. These latter nodules consist of epithelioid, oval and round cells, derived partly from proliferation of the capsular endothelium, partly from other fixed cells, and partly from the blood-vessels, and accumulated around and eventually invading and displacing the ganglion cells. In their first publications these authors believed that they had found the long sought specific lesion of rabies, but van Gehuchten is now of the opinion that this lesion characterizes only the natural disease in man and animals, and is either absent or only imperfectly manifested in the experimental disease, at least by the ordinary modes of inoculation, whether by virus of the streets or fixed virus. The ganglionic lesion is more marked in dogs than in man, and least developed in rabbits.

The findings of van Gehuchten and Nelis have been in the main confirmed by Hébrant (47), Nocard (48), Cuillé and Vallée (49), and

Ravenel and McCarthy (50), so far as the constancy of the lesion in dogs dead of the natural disease is concerned. The search for the lesion in experimental rabies has given apparently conflicting results. Whereas Ravenel and McCarthy obtained positive results even in rabbits dead of subdural inoculation with the street virus, van Gehuchten (in his later publications) and Hébrant were unable to find the lesion either in rabbits or in dogs after subdural inoculation, these latter animals, as already stated, presenting the most advanced and characteristic changes after death from the spontaneous disease. There are not as yet sufficient observations to judge of the frequency of the lesion in human beings. It was well marked in a case reported by Gratia (51), but was absent or at most only slightly apparent in Sano's (52) patient.

It appears from later investigations that the ganglionic lesion described by van Gehuchten and Nelis is not, as they at first supposed, specific for rabies, but may occur in other diseases. Babes, who protests vehemently against the exclusive position taken by van Gehuchten and Nelis, says that he has met and described this alteration in several diseases, and essentially similar changes have been recently reported in various affections by Crocq (45), de Buck and de Moor (53), and Spiller (54).

It would appear, therefore, as stated by Crocq, that, while no lesion specific for rabies has hitherto been discovered, the totality of the lesions, both those emphasized by Babes and those described by van Gehuchten and Nelis, makes a picture sufficiently characteristic to warrant usually the diagnosis of rabies, when all of the circumstances of the case are considered. The absence of these lesions does not, however, exclude rabies.

MATERIAL AND METHODS.

The material used in the present study of the nerve-cell changes in rabies was obtained from nine rabbits which had been inoculated with fixed virus, an emulsion made from the medulla of a rabbit from a previous series being used. The skull was trephined and the injection was made under the dura. The strength of the virus was such that it proved fatal to control rabbits in from seven to nine days.

Rabbit No. 1. Killed by chloroform three days after inoculation. This rabbit showed no symptoms, was eating well and was apparently in a perfectly normal condition.

Rabbit No. 2. Killed by chloroform four days after inoculation, on

first sign of symptoms. These consisted merely in slight disinclination to eat and in lessened activity.

Rabbit No. 3. Killed by chloroform five days after inoculation. Partial paralysis for about 24 hours.

Rabbit No. 4. Killed by chloroform six days after inoculation. Partial paralysis for about 36 hours.

Rabbit No. 5. Died six days after inoculation. Was taken sick much earlier [second day] than the other rabbits and differed from them in showing much greater activity.

Rabbit No. 6. Died on seventh day after inoculation. Paralysis was almost complete. Had showed symptoms for about 60 hours.

Rabbit No. 7. Died on eighth day, four days after initial symptoms. Paralysis complete.

Rabbits Nos. 8 and 9. Died on ninth day. Paralysis complete.

The rabbits belong to two series, rabbits Nos. 2, 4, and 9 belonging to one, while rabbits 1, 3, 5, 6, 7, 8 belong to a second series. Most of the material was fixed in van Gehuchten's fluid (see p. 557) followed by strong alcohol. In some cases alternate segments were fixed in formalin 10%, followed by graded alcohols.

The sections were of a standard thickness of 6μ. For special purposes a few thinner sections were made as noted. The staining was mainly by the erythrosin-methylene-blue method described on page 558. A few sections were stained with methylene blue alone.

PERSONAL OBSERVATIONS.

Rabbit No. 1.—*Spinal Cord and Medulla.* There is a marked congestion of both grey and white matter throughout the entire cord and medulla. There are no changes in the vascular walls, no exudation, hæmorrhages, or increase of neuroglia.

Cells of the anterior horn.—The majority of the spinal stichochromes appear perfectly normal. Of cells which show departure from the normal, we observe:

1. Cells, with normal nucleus and cytoreticulum, in which the chromophilic bodies, although normal in size and shape, present ragged, frayed-out edges, an appearance quite different from that of the chromophilic bodies found in the normal cell. There is no breaking up of the bodies, no lessening of intensity of staining, no diffuse staining of the cell protoplasm (Plate XXXVI, Fig. 10, a).

2. Cells with normal cytoreticulum, in which the chromophilic bodies, while normal in size and shape, in addition to the ragged appearance mentioned, take a much paler stain (Plate XXXVI, Fig. 10, b). In these cells the nucleus and nuclear contents remain normal, or the nucleus is somewhat swollen. Whether normal in size or swollen, its reticulum is apt to show an increase in the thickness of its strands, which thus appear more sharply defined. The same is true of the nuclear membrane, which thus gives to the nucleus a more distinct contour.

3. Cells in which, with or without the above nuclear changes, there is a marked reduction in the chromatic element.

(a). The Nissl bodies may retain their normal size and shape, but, in addition to the roughness of outline, appear full of ragged holes or vacuoles. On closer examination these are seen to correspond to the meshes of the cytoreticulum (Plate XXXVI, Fig. 10, c).

(b). Through further reduction in the chromatic substance the shape of the Nissl bodies is lost, and the basophilic element appears as fine granules incrusting more or less thickly the strands of the acidophile reticulum. At nodal points of the reticulum there are apt to be larger accumulations of granules. Not all strands of the reticulum are covered with the granules, nor do those covered have an equally thick incrustation at all points. The result is often to give the cell the appearance of having two distinct reticula, a fine meshed, acidophile reticulum, and a much coarser meshed, irregular, basophile reticulum. The chromatic rods or spindles in the dendrites, and in the cones which mark the dendritic bifurcations, do not show the same tendency to disintegration as the chromatic masses of the cell body proper. Even in those cells in which chromatolysis was most marked, these spindles and cones retained their smooth outline, finely granular appearance, and normal staining qualities. Such cells are found throughout the entire cord and medulla. The changes are, however, more pronounced, and involve a larger number of cells, in the cervical and upper dorsal, than in the lower dorsal and lumbar regions.

The stichochromes of the *medullary nuclei* show a larger propor-

tion of affected cells, and a larger proportion of these show greater loss in chromatic substance, than the cells of the cord. In the lower part of the medulla, where the grey matter of the anterior horns becomes broken up by decussating fibres, extremely large cells, of the type of the anterior-horn cells, are found scattered singly or in small groups. A large proportion of these cells are affected, and many show an almost complete disappearance of the chromophilic bodies. There is no selection of cranial nuclei, all being quite evenly affected; nor is there in the cord any tendency to degeneration of cells by groups; cells in the various conditions described lying in close proximity to one another and to normal cells. Speaking generally, those cells lying in the ventro-lateral portion of the horn, near the margin of the grey matter, show least changes, while those lying deeper and more internal, are most affected. Plate XXXVIII, Fig. 22, shows one of the more changed cells from the deeper part of the grey matter, near the central canal, in the upper cervical margin. Of changed cells, those described under 1 are much the most numerous, while those under 2 and 3, a and b, are in the order of decreasing frequence.

The cells of the *spinal ganglia* show no variation from the normal.

The *cells of Purkinje* show nothing beyond the usual wide variation in quantity and arrangement of the chromatic substance.

In the *cortex* and in the mitral cells of the *olfactory lobe*, no changes could be discovered.

Basal ganglia.—In the nuclei of the basal ganglia, the changes resemble closely those described in the spinal cord, corresponding as regards number of cells affected, and extent, to the upper or middle cervical region. There seems to be a decrease in intensity of the lesion as we pass in either direction from the medulla oblongata. In the larger ganglion cells scattered throughout the nucleus caudatus and the nucleus lenticularis, and in those making up the bulk of the postero-lateral nucleus of the thalamus, the changes are identical with those described in the spinal stichochromes.

Rabbit No. 2.—*Spinal Cord and Medulla.* The congestion is more marked than in the preceding animal, and extends throughout

cord and medulla. The walls of some of the vessels are infiltrated with leucocytes, sometimes many and sometimes few. These vessels may show also an increase in the connective tissue elements of the wall, most marked in the small vessels of the grey matter, the walls of which are normally very thin. Less frequently the latter condition is found without the leucocytic infiltration.

Hæmorrhages are common both in grey and in white matter. They are usually small, but sometimes are extensive, especially those in the grey matter of the anterior horn. They are always without any distinct limitation.

There appears to be no direct relation between the vascular changes and the cellular lesion. Normal cells are as common in the areas of most intense congestion and in the vicinity of vessels in the walls of which the most changes are found as elsewhere. I have seen apparently normal cells lying in the midst of a large hæmorrhage.

Cells of the anterior horn.—The cellular lesion is much more pronounced than in Rabbit No. 1. There are fewer normal cells, although these are still in the majority. The number of cells showing the lesser degrees of change, as described for the preceding cord, is increased. There is the greatest relative increase, however, of those cells which show the more marked chromatic changes.

The nucleus and nuclear contents are normal, or show only those slight changes already described. By far the majority of altered cells present only the lesser degrees of change already described, but there is often a much more diffuse granular blue staining. This obscures the cytoreticulum and gives the cell a quite homogeneously granular appearance. The cytoreticulum in those cells in which it is not lost in the diffuse blue granulation, appears normal.

A few cells show evidence of a more advanced condition of degeneration. Such cells, found in the cord, but more commonly in the medullary nuclei, or in the remnants of the anterior horn, show the following changes: For convenience of reference we will designate them as belonging to type 4, the changes already noted in Rabbit No. 1 having been referred to types 1, 2, and 3, a and b. The nucleolus, sometimes normal, is more often roughened at its edges, some-

times small pieces being broken off from the main mass. The intranuclear network is broken up, often appearing only as a stellate mass of granules around the nucleolus and as little thread-like projections from the inside of the nuclear membrane. The nuclear contour may be very distinct, and with its normal spherical shape; less commonly it is irregular, or somewhat crenated. There may be a break in the continuity of the nuclear membrane, in which case the nucleolus, though usually remaining within the nucleus, may be outside what appears to be the nuclear limits.

The Nissl bodies are broken up into small fragments, which usually lie upon nodal points of the reticulum. Between the fragments there is a fine, blue, granular deposit. The larger fragments may be absent and only the fine granules remain. The chromatic rods in the dendrites retain their normal appearance. The cytoreticulum, even in these cells, appears normal. The entire cell is often swollen, sometimes sufficiently to exert pressure upon the surrounding structures. More rarely it is shrunken, leaving an exaggerated pericellular lymph space.

The distribution of the lesion corresponds to that given for Rabbit No. 1, except that there is less difference between the upper and the lower regions of the cord. As in No. 1, the medullary nuclei and the cells of the anterior horns are especially affected.

The cells of the *spinal ganglia* are normal.

In the *cerebrum* and *cerebellum* the congestion is less marked than in the cord, and the changes in the vessel walls are absent.

There is a decided reduction in the average amount of chromatic substance in the cells of Purkinje, but many appear normal. Nuclear changes are seen in but few cells, and these are of the milder grade, as described in Rabbit No. 1. Some cells of Purkinje show a considerable amount of chromatic substance, but in a much finer state of subdivision than normal; others show complete, or almost complete, loss of the basophile element, with a diffuse blue ground-substance, a finely granular blue ground-substance, or the same with a few large granules. Both their behavior and their relative numbers would indicate that the former change is a result of degeneration of those

Purkinje cells which are normally rich in chromatin; while the latter results from changes in cells which are normally deficient in this constituent.

No changes were found in the cells of the cerebral cortex, or in the mitral cells of the olfactory lobe.

Basal ganglia.—The large stichochromes of the nucleus caudatus and the nucleus lenticularis, and the similar cells found in the nuclei of the thalamus, present the same degrees of degenerative changes as described for the spinal stichochromes. Rather more cells are affected, and the lesion of the individual cell is somewhat more advanced than in Rabbit No. 1. In the smaller ganglion cells there is an evident decrease in the basophile element. These cells differ from one another so greatly, as regards their normal content of basophile substance, that it is difficult to say of any particular cell that there is a diminution. When, however, the chromatic substance of a number of cells is estimated, it is apparent that the total average amount is considerably decreased.

Rabbits Nos. 3 and 4.—The lesions found in Rabbit No. 3 and in Rabbit No. 4 resemble each other so closely that they may be described together. No. 3 was killed on the 5th and No. 4 on the sixth day after inoculation. They belong to different series (p. 585).

Spinal Cord and Medulla.—There is an increase both in the congestion and in the leucocytic infiltration of the walls of the blood-vessels. These leucocytes may form an even ring around the vessel, or, more commonly, there is a large collection of them at one or two points in the vascular walls. There seems to be no advance in the increase of connective-tissue elements, that feature remaining the same as in Rabbit No. 2. Hæmorrhages also are about as frequent as before.

Lesions of the nerve cells show a marked advance. Normal cells are still found, but in decided minority, so that search through several sections at any level of the cord may be required in order to find an entirely normal cell. They are still present in those regions where they have already been described as most frequent, namely, in the outer and ventral corner of the horn, and with decreasing frequency

from below up. All of the cellular degenerative conditions, previously described, are present, and are increasingly frequent. The relative increase is, however, greatest in cells showing the more extensive changes. Thus cells of type 3 a and b (p. 586) and type 4 (p. 588) are now quite numerous. Of further changes the following were noted:

Nucleus and nuclear contents.—(a). The nucleolus is usually rough, stains more irregularly than heretofore, appears rather coarsely granular, and is often broken up into several pieces (Plate XXXVI, Fig. 11).

(b). The nuclear reticulum is in many cells abnormally distinct and darkly stained, with larger mesh openings, due to a coalescence of some of the strands of the reticulum. This may stand out against a perfectly clear background, or one in which there is more or less granular or homogeneous pink color. There may be no sign of a reticulum, the entire nucleus staining a homogeneous, an evenly granular, or an irregularly granular pink color. The nucleus may assume almost any shape, is commonly very much shrunken and crenated, when it appears as an irregular red mass surrounding the nucleolus (Plate XXXVI, Fig. 11).

(c). The nuclear membrane is often broken, sometimes showing only in spots, again having completely disappeared; in this case the nucleolus appears to lie in the general cell protoplasm, with or without some remnants of the acidophile nuclear elements attached to it. In any of these conditions the nucleus may be eccentric.

Cell body.—(a). The basophile element of the cell body shows all of the types of changes already described. The most frequent condition is that described in Rabbit No. 1, as type 3, a and b, in which the basophilic granules appear as a precipitate upon the strands of the cytoreticulum. This gives the appearance of a densely granular, blue network with coarse strands and small mesh openings (Plate XXXVI, Fig. 11). In places where the granules are absent, or thin, the acidophile reticulum shows plainly. In contrast to those cells described in Rabbit No. 1, type 3, b, in which the chromatic substance is present in fine granules, and the chromatic spindles and cones in the

dendrites are intact, these cells in which the chromatin is arranged as a network, rarely show any normal condition of the dendritic chromophilic bodies, these being usually absent (Plate XXXVI, Fig. 11).

(b). The cytoreticulum appears intact, excepting in cells where there is considerable diffuse staining. In such cases it seems probable that the reticulum is obscured rather than destroyed.

(c). The outline of the cell body is often broken and irregular. The cell is more commonly shrunken than swollen or normal in size. These cells have the usual number of protoplasmic processes, many of which, however, are shrunken, stain dark red, and end in a point at a short distance from the cell body. Plate XXXVI, Fig. 11, illustrates quite typically several of the points described.

The distribution of the lesion is more uniform than before throughout the cord and medulla, being almost as extensive in the lower as in the upper cord and in the medulla.

The cells of the spinal ganglia show no changes.

Purkinje's Cells.—These are much more altered than in the preceding rabbits.

Nucleus and nuclear contents. (a). The nucleolus is usually ragged, sometimes broken up into several pieces. Eccentricity is often extreme, in some cases the nucleolus lying on the nuclear membrane.

(b). The nucleoreticulum is more or less completely disintegrated, and represented by clumps of granules surrounding the nucleolus and attached to the nuclear membrane. These granules are much coarser than those resulting from the disintegration of the nucleoreticulum in the anterior horn cells. There is almost no tendency to diffuse staining of the nucleus, as seen in some anterior horn cells.

(c). The nuclear membrane is in most cells very sharply defined and darkly stained, and preserves its spherical shape: more rarely it is crenated or broken or both. These latter conditions are not nearly so frequent as in the spinal stichochromes of this same rabbit.

Cell body. The various conditions of the cell, as regards the chromatic elements, are quite similar to those described in the cord and medulla, bearing in mind the normal differences. There are

some normal cells. A considerable number of cells show complete chromatolysis, the cytoreticulum being very distinct and apparently normal. Between this and the norm all grades exist. Two features of the chromatolytic process observed in the anterior horn cells, were absent in the cells of Purkinje. First, there was no appearance of fraying out of the edges of the Nissl bodies, as is the case in the anterior horn cells; they seem rather to disintegrate, breaking up into smaller, but no less dense and smoothly outlined masses. Second, there is no such distinct formation of a basophile network as in some of the anterior horn cells.

Staining of the ground substance is extremely irregular; it may be clear, or blue and granular, or homogeneous. There may be no blue staining whatever, the cytoreticulum then appearing very distinct against a clear background, or all or part of the cell may present a granular or homogeneous pink appearance. The cell contour is rarely broken, but may be irregular or shrunken.

Cerebral cortex. The congestion is not marked as in the cord; some of the vessel walls show exudation of leucocytes, but this also is less marked than in the cord.

The distribution of the cortical lesion is uniform, all types of nerve cells in all situations showing modifications. In the smaller pyramidal cells, and especially in those least rich in chromatic substance, earlier changes are difficult to study. In larger cells, both pyramidal and polymorphous, there is a disappearance of the larger masses of chromatic substance which lie upon the nodal points of the acidophile reticulum, with a resulting added clearness of the network-appearance of the basophile granules which lie upon the strands of the reticulum. Plate XXXVIII, Fig. 23, illustrates some of the conditions found in these cells. The changes in the smaller pyramidal cells are the same as in the larger; disappearance of chromatin is apt to be more complete. The large polymorphous cells of the deeper layers of the motor areas of the cortex show about the same degree of changes as those described in Rabbit No. 1 for the anterior horn cells. Few show more than a raggedness in the outline of the chromophilic bodies.

Mitral cells of the olfactory lobe. Most of the cells show changes. It will be remembered that extremely large, finely granular, dense

chromophilic masses arranged parallel to the nuclear outline and to the surface of the cell, are characteristic of these cells (p. 573), the nuclear cap being usually very large. These masses are usually packed closely, leaving few unstained portions of the cell, and largely obscuring the cytoreticulum. In most of the cells in Rabbits Nos. 3 and 4, these large chromophilic bodies are entirely or partly broken up into various sized masses and granules, distributed unevenly throughout the cell body. Some cells show no large Nissl bodies; others show but one or two, while the rest of the cell body is occupied by small masses and fine granules. The acidophile reticulum, which in the normal cell is barely visible between the dense chromatic masses, is now quite clear. There is little tendency to diffuse staining of the cell background (Plate XXXVI, Fig. 12).

The nucleolus is usually ragged or broken up. The nuclear reticulum is often somewhat disintegrated, though its strands are often quite distinct. The outline of the nucleus is smooth, rarely irregular, and the nucleus remains approximately in the centre of the cell.

In the case of all the cellular lesions previously described, speaking generally, there is some relation between the extent of nuclear changes and that of alterations in the body of the cell. Thus the more advanced nuclear changes are found usually in cells in which the greatest changes have taken place in the chromatic elements. This is, however, not always the case. Advanced nuclear disintegration is rare in cells showing mild grades of chromatolysis. On the other hand it is not at all uncommon to find a nearly normal nucleus in the midst of marked changes in the cell body.

Basal ganglia. Few of the larger cells of the nuclei of the corpus striatum and thalamus are normal. The extent of the lesion corresponds almost exactly to that in the upper part of the cord, both as regards the number of cells affected and the character of the lesion in the individual cells. Many of the cells present the condition illustrated in Plate XXXVIII, Fig. 22. The smaller cells show about the same extent of chromatolysis as in Rabbit No. 2.

Rabbits, Nos. 5 and 6.—*Spinal Cord.* There is less congestion than in any of the preceding cases. There is no change in the small round-cell infiltration of the walls of the blood-vessels.

Cells of the anterior horn. All of the previously described types of degeneration are found. It is still possible to find normal cells, though they are rare; none were found except in the extreme ventral and lateral region of the horn. Of altered cells, few show the earlier changes with normal or slightly changed nuclei and with simply frayed-out chromophilic bodies. More extreme degrees of change, as shown in Plate XXXVI, Fig. 13, are the more common.

Nucleus and contents. The nucleolus, in addition to the changes already described, may be completely disintegrated and represented only by a few fine or coarse blue granules scattered throughout the nucleus.

The nuclear reticulum has, in the majority of cells, disappeared, the entire nucleus taking a granular or diffuse pink stain.

The nuclear membrane is often extremely distorted and broken, sometimes absent. In cells showing more marked forms of degeneration, there may be no trace of nucleus or nuclear contents found, this being determined of course by serial sections. Such apparent absence of the nucleus is most common in cells in which the protoplasm takes a dark stain; while in those cells in which there is a lighter stain, remnants of the nucleus are always found. It would seem probable that the disintegrated nuclear elements, basophile and acidophile, are simply indistinguishable among the similar elements of the cell body proper, when both are in a fine state of disintegration. Eccentricity of the nucleus, while found, is not a common occurrence.

Cell body. Of the conditions already described, the most common is that in which the basophile elements are arranged in the form of a reticulum upon the acidophile network, as described in Rabbit No. 1, type 3 (Plate XXXVI, Fig. 11). In most of these cells, however, the network is much less regular. We may note also the following:

1. Cells in which there is apparently complete disintegration of all stainable elements, both acid and basic. These cells take on a somewhat different appearance according to the greater intensity of the acid or basic stain. With erythrosin alone they take a strong, homogeneous, or granular red stain, with or without scattered, larger, brighter, red granules. With methylene blue alone, they present the

same picture, substituting the blue for the red. With a double stain, they may present a homogeneous appearance, usually of a violet color, the tint inclining towards the red or towards the blue, according to the overstaining. The majority of such cells, however, are not homogeneous, but granular, in which case it is always possible with a proper staining to differentiate red granules from blue granules; the one evidently representing the disintegrated remains of the acidophile reticulum, the other, of the basophilic elements of the cell. In most such cells, the protoplasmic processes are shrunken and irregular in outline. In many, some or all of the dendrites are traceable only a short distance from the cell body, ending usually in points, or in little shrivelled strings. These shrunken, broken processes stain considerably more intensely with erythrosin, than does the cell body to which they belong. They may show a few small remnants of the chromatic spindles; more commonly, however, they present nothing but a homogeneous or granular red appearance (Plate XXXVI, Fig. 11, and Plate XXXVI, Fig. 13, a). There is no evidence of any reticulum. Such cells are usually normal in size, or swollen. Their outline may be distinct and smooth, very indistinct, or, less commonly, broken and ragged.

2. A very common condition of the cell body, more common than that just described, corresponds partly to that noted in Rabbit No. 1, type 3. The basophile element is not greatly reduced. In parts of the cell, commonly the peripheral, it is present as a network though much broken up (Plate XXXVI, Fig. 11); in other parts, it is distributed as irregular, granular masses, apparently situated on nodal points of the acidophile reticulum, the larger covering several meshes. In addition are present, often in clumps, long slender blue rods. These are very narrow, straight or wavy, stained intensely, and appear to have no relation to the acidophile network (Plate XXXVI, Fig. 13, b). They give to the cell a quite characteristic appearance. In such cells the acid reticulum usually remains intact, at least in a portion of the cell. The cell body is apt to be shrunken. The dendrites, if shrunken at all, are only slightly so, and, as a rule, contain chromophilic bodies, somewhat broken up. The cell outline is often

ragged and indistinct. Such cells are most common in the periphery of the anterior horn, and represent the larger cells.

Spinal ganglia. Cells of the posterior root ganglia at all levels of the cord, show changes, with no differences in their extent for different levels. The *nucleus* may be in any of the conditions already described in the case of cells from other regions. In those cells in which there is distinct central chromatolysis, the clear area, just outside the nuclear membrane, brings the nucleus out in strong relief. In other cells the outline of the nucleus is broken completely or lost. An extremely common condition is a crenated nucleus somewhat shrunken, taking a diffuse granular pink stain, containing an indistinct rough-edged nucleolus. Eccentricity, sometimes so marked as to cause bulging of the cell periphery, is not uncommon (Plate XXXVII, Fig. 16, e).

. *Cell body.* 1. There are cells which in the nature of the change resemble the anterior horn cells, type 1, Rabbit No. 1 (p. 585). They show the normal number, size and arrangement of chromophilic bodies, but these have frayed-out, feathery edges.

2. A very common condition in these cells is central chromatolysis (Plate XXXVII, Fig. 16, d, e). The central portion of the cell may be pale blue, with or without very fine blue granules scattered through it, or perfectly colorless. The extent of the chromatolysis varies from a small area immediately around the nucleus, to an involvement of most of the cell, leaving but a narrow rim of chromophilic bodies at the extreme periphery. In the latter case, these bodies are usually large, very dark, run into one another, lie parallel to the outline of the cell body, as if they were being pushed out by centrifugal pressure (Plate XXXVII, Fig. 16, b).

3. A peculiar condition noted in a few cells, consists in a perfectly clear background with a continuous or a broken cytoreticulum, and, scattered quite evenly through it, rather small chromophilic bodies, which are smooth in outline and seem perfectly homogeneous (Plate XXXVII, Fig. 16, c).

The cells of Purkinje show a marked progression of the lesion as compared with the preceding specimens. They are diminished in

number. In cells arranged in distinct rows, Purkinje's cells and the mitral cells from the olfactory lobe, there is a fairly even frequency for a given thickness of section. Thus comparing a 6μ section of the present specimen with a 6μ section from a normal rabbit, a marked loss of cells is easily recognized. Few cells are normal. Many are found which present the picture described for the Purkinje cells of Rabbits Nos. 3 and 4. A majority, however, are in more advanced conditions of degeneration, as shown in Plate XXXVII, Fig. 17.

In these degenerated cells the nucleus may present a great variety of appearances. It may be irregular in outline. The intra-nuclear network may be normal, or it may show in a part of the nucleus, while in the remainder it is represented only by reddish granules. The nucleus may have no distinct outline, but appear simply as an area of pale pink granulations (Plate XXXVII, Fig. 17, c). It may be a shrunken red mass, as in Plate XXXVII, Fig. 17, d. In most of the nuclei there was a coarse blue granulation (Plate XXXVII, Fig. 17, b, c, d). In some, this appeared to be due to a disintegration of the nucleolus; in others, it seemed equally extensive without any reduction in the size of the nucleolus.

In the bodies of most of the cells there is extremely little chromatic substance. When present it is as short threads or rods, or as small granular masses. The reticulum in these cells is indistinct, showing, if at all, only at the periphery of the cell (Plate XXXVII, Fig. 17, a). The remainder of the cell body often has a granular pink appearance. The outline of the cell is in many cases irregular and the processes are indistinct.

Cerebral cortex (Plate XXXVII, Fig. 18). The changes in the cortical cells show a considerable advance, as may be seen by comparison of Figs. 18 and 23. It should be noted that only the drawing of a very large number of cells could give any adequate picture of the shades of variation which these cells present. Many cells still remain normal; these, however, are much less frequent than in the cortices of the preceding rabbits, and more of the changed cells show the more extensive modifications. Plate XXXVII, Fig. 18, b, shows one of the less affected cells, which resembles quite closely those seen

in Fig. 23. The nucleus is distorted. In cell, a, of the same figure there is considerable shrinkage of the cell body and but little evidence of the reticular arrangement of the granules. The nucleus is irregular, eccentric and only a few shreds of its reticulum remain. In c of the same figure the loss of basophile substance is complete, leaving an extremely clear demonstration of the apparently unchanged acidophile reticulum. As in rabbits 3 and 4 there is no special localization of the cerebral lesion, the cells being affected to an equal extent in all regions.

Mitral cells of the olfactory lobe (Plate XXXVII, Fig. 19). Even more marked than in the cells of Purkinje is the progress which the lesion has made in the mitral cells. As in the cerebellum, there is a decided and even a greater reduction in the number of cells. One can in places pass along the line of mitral cells for several fields of a No. 7 lens without seeing a single mitral cell. The least changed cells show the outlines of some of the larger chromophilic bodies. In cell a of Fig. 19 most of the large chromatic masses are broken up and represented by rather small collections of granules; where the larger chromophilic bodies remain, they are ragged and fenestrated. The cytoreticulum in cells which are of about normal size, is usually quite distinct. In Fig. 19, are seen two of the least changed cells from the olfactory lobe of Rabies Rabbit No. 5. They are in many respects similar to those in Plate XXXVI, Fig. 12 of Rabies Rabbit No. 3. The disintegration of the chromophilic bodies is more extensive and the actual amount of basophile substance is less. In the dendrites the basophile granules are usually arranged as fine straight, or more often wavy lines and as oval masses in the varicosities; in this way they often project somewhat from the surface like knots on a branch of a tree, giving the process a rough, wavy appearance. In these cells the nucleus is apt to be distorted and the nucleo-reticulum more or less disintegrated.

Smaller cells are found, which are shrivelled and irregular in outline, and with little appearance of structure remaining. The nucleus is represented usually by a shrunken red or purple mass around the remains of the nucleolus. Broken parts of cells, pieces of cell

bodies and of dendrites, are seen scattered along the line occupied by the mitral cells.

Basal ganglia. The large cells of the corpus striatum and of the thalamus present much the same appearance as the cells of the anterior horn (Plate XXXVI, Fig. 13). A considerable number of cells show a rather extreme condition of chromatolysis giving them the appearance depicted in Plate XXXVIII, Fig. 22. The network arrangement of the granules is not so frequent as in the cells of the cord. Vacuoles of various sizes in the cell body seem to be of rather more frequent occurrence than in the anterior horn cells. The loss of basophile substance in the smaller cells has become more pronounced. Few of the cells show any of the large blue masses; most show only a fine blue granulation, some an almost complete chromatolysis.

Rabbits Nos. 7, 8, and 9.—These rabbits, one dying on the eighth day and the others on the ninth day after inoculation, showed such similarity in the lesions, that they will be discussed together, any individual peculiarities being noted.

Spinal Cord and Medulla.—Congestion is present and quite intense in Rabbit No. 8, very slight in Nos. 7 and 9. It is uniform throughout cord and medulla.

Hæmorrhages, mostly in the grey matter, and at different levels of the cords, are frequent in all three rabbits. They occur (a) into the grey matter without definite limitations; (b) into the peri-vascular lymph spaces, and (c) into the peri-cellular lymph spaces around nerve cells, as shown in Plate XXXVII, Figs. 20 and 21, the nerve cell being pressed by the blood to one side of the space. Plate XXXVII, Fig. 21, shows a blood-vessel and its relation to the cell space, and peri-cellular hæmorrhage. In several instances the plane of the section was such as to make a peri-cellular hæmorrhage appear as if situated inside the cell itself, entirely surrounded by cell substance.

Changes in the walls of the blood-vessels are not nearly so pronounced as in some of the rabbits killed earlier in the series. Only a few vessels, mainly in the medulla, show slight infiltration of their walls with leucocytes. The increase in connective tissue elements is also not common, and usually only a few new cells are present.

Cells of the anterior horn (Plate XXXVIII, Fig. 24, and Plate XXXVI, Fig. 14). No normal cells were observed. With the exception of the very earliest stage, in which there is little else than a ragged or somewhat ragged appearance of the outlines of the chromophilic bodies, all of the types of degenerative condition already described are present. The great majority of the cells, however, fall into one of the two following classes:

1. Cells in one respect similar to those already described in which there is complete or almost complete chromatolysis. They show certain further changes which appear to mark the progress of the degenerative process.

(a). The *nucleus* is usually irregular in shape; its membrane may be distinct, thickened, and stained a brilliant red. Within this nucleus there is no distinct reticulum, but a larger or smaller number of round or irregular, apparently homogeneous masses, staining bright red, and most numerous around the nucleolus. Some of these masses are often as large as the nucleolus itself. The apparent total amount of this acid-staining nuclear substance, is often much greater than in the normal cell, and is difficult to explain in any other way than by supposing an absolute increase in the acidophile elements of the nucleus. Instead of these distinct red globules, the entire nucleus may be simply an irregular bright red mass, with or without a nucleolus, and with or without a visible enveloping membrane. There is sometimes no nuclear limitation, a nucleolus surrounded by a few bright red granules, or the red granules without the nucleolus, lying loose in the general cell protoplasm. Less commonly there is entire disappearance of the nucleus.

(b). The *cell body* shows no evidence of chromophilic bodies or of a cytoreticulum. The acidophile portion of the cell body is represented by a homogeneous, or finely granular pink or bright red background. There is considerable difference in the intensity of the stain in different cells and in different parts of the same cell. Speaking generally, the stain is lightest in the central portion of the cell, and grows darker as the processes are approached. The processes themselves are much darker than the cell body proper, this being especi-

ally true of the axis cylinder process and its axone hillock, which often stands out with an extremely bright red stain. The protoplasmic processes have, like the cell body, lost all trace of a reticular appearance, but often show an indefinite longitudinal striation. There may be no signs of the blue-staining element of the cell; or there may be some coarse blue granules standing out sharply against the red background; or there may be a very fine blue granulation, giving the cell a diffuse violet or purple color (Plate XXXVIII, Fig. 24). The same thing impresses one in regard to the acid elements of the cell body, as has already been noted in regard to the acid elements of the nucleus, namely, a very decided increase, or at least an apparent increase in these elements. When we remember how very delicate and lightly staining are the strands of the cytoreticulum in the normal cell, it seems necessary to think of either an absolute increase in the amount of acidophile substance, or else that, occurring in a different condition, as diffuse granules or dissolved in the general cell protoplasm, it so takes up the red stain as to appear present in greater quantity. Such cells are usually not shrunken, but entirely or almost entirely fill up the cell spaces. Their dendritic processes are reduced in diameter, and often taper rapidly to a point but a short distance from the cell body. They contain little or no chromatic substance.

2. Cells in which there is a considerable amount of chromatic substance, and this substance deposited in quite a characteristic manner.

(a). The *nucleus* in these cells is often perfectly round, with a broad, bright red nuclear membrane and is usually in the centre of the cell. There is no distinct intra-nuclear network. The most common condition within the nucleus is that already described—an apparent increase in the acidophile element, which is present as round or irregular masses of considerable size. More rarely the nucleus is crenated and may be in any of the conditions previously described.

(b). In the *cell body* an imperfect cytoreticulum is often visible in some parts, the remainder of the cell showing a diffuse pink background, homogeneous, or more often finely granular. As in the previous type of cell, the processes show a stronger red than the cell body, but in this type they usually contain some remnants of the

chromatic spindles. Moreover, they do not taper off at so short distances from the cell as do those of the preceding type.

It is in the chromatic element that these cells show their peculiarity. It will be remembered that in Rabbits Nos. 5 and 6 (Plate XXXVI, Fig. 13, b), was noted the occurrence of a part of the chromatic substance as dense thin rods, straight or wavy, and taking a very dark homogeneous stain. Here there has been both a decided increase in the number of cells in which the chromatic substance is deposited in this manner, and an increase in the proportion of chromatic elements thus deposited. The remaining chromatic substance is present as irregular masses, some small, some showing the general shapes of large normal Nissl bodies, but full of vacuoles and with edges which, instead of being granular, appear hard and sharp cut. These edges and the edges of the vacuoles take a very dark homogeneous stain. Among these are found a few chromophilic bodies of the usual granular types, taking by contrast a rather pale blue stain. It is impossible to tell whether there is in such cells a loss of chromatic substance, and if a loss, how extensive. It would appear rather as if there was a contraction or shrinkage of the more loosely aggregated finely granular chromophilic bodies into the hard dense masses seen in these cells.

Spinal Ganglia (Plate XXXVI, Fig. 15). The changes resemble those already described in Rabbits Nos. 5 and 6. More cells are affected, there being none, apparently, that are normal.

The nucleus is usually more or less shrunken or crenated and is often eccentric. It may correspond to the nuclear condition just described in anterior horn cells, in which there is an apparent increase in acidophile elements, these being present as larger or smaller discrete masses or even distributed so as to give a diffuse red stain to the entire nucleus. The nucleolus is in most cases round and smooth and much the same as in the normal cell.

The basophile element of the cell body is somewhat more reduced than in the preceding cases, though not markedly. That condition described as type 3 in the ganglion cells of Rabbits Nos. 5 and 6 (p. 597), is more frequent. These cells in which the bright blue bodies are small, sharp-edged, smooth, quite homogeneous, and evenly

distributed through the clear or pink cell body, present quite a characteristic picture.

The cytoreticulum is in most cases entirely indistinguishable. When visible, it is usually around the periphery of the cell, and even then much disintegrated. The general cell body takes a diffuse stain, varying from a light pink to a pronounced red. It is homogeneous, or, more rarely, finely granular. Vacuoles are frequent, often very large, one sometimes occupying a quarter of the cell body. Distinct disintegration of the cell body is not uncommon, when all that remains of the cell is a shrivelled mass of pinkish staining substance, with or without a few small blue masses, hung by thread-like strands to the edges of the cell space (Plate XXXVI, Fig. 15, b).

Cells of Purkinje. As compared with Rabbits Nos. 5 and 6, there seems to be no further diminution in number of these cells, nor do they show any further loss of chromatic substance. The nucleus is oftener irregular and crenated, but as a rule it presents a distinct, coarsely granular network and membrane, and a normal nucleolus. It is often eccentric, sometimes lying on the edge of the cell. The outlines of the cell body have also become more irregular, often shrunken. There is a distinct reticulum. Stained with erythrosin alone, this presents a granular red appearance; with methylene blue alone, a granular blue; with the double stain, a granular violet or purple. Around the periphery of the cell, it was possible to make out, in double stained specimens, a few strands of the reticulum, stained red and uncovered by blue granules; it seems probable, therefore, that in the central areas, the same condition obtains as in the stichochromes of the anterior horn, namely, an acidophile reticulum incrusted with fine basophile granules. In these cells, however, a satisfactory demonstration of this could not be secured.

The main protoplasmic process and its branches are apt to be wavy and knobbed. They contain much of the basophile substance in the form of fine granules, often strung along in rows. The cone of bifurcation of the first division of the main dendrite often preserves its general outline, while having a ragged eaten-out appearance.

Cerebral cortex. The cerebral congestion was not marked in any of these three rabbits, nor were the vascular changes at all extensive.

As was the case with the cells already described from the anterior horn, medulla, spinal ganglia and cerebellum, there was little apparent progress in chromatolysis of the cortical nerve cells. There are many normal cells, that is, many which contain a relatively small amount of chromatin, arranged as a network and without much thickening at the nodal points. There are few, however, of those cells found in the normal cortex, which are rich in chromatin, having large nodal masses and chromophilic bodies which cover over several of the meshes of the reticulum. It seems probable, therefore, that many of the apparently normal cells represent cells which normally would have a larger amount of chromatin, and in which the chromatolytic change has resulted in loss of the larger chromophilic bodies, the chromatic network remaining. The probability of the correctness of this deduction is enhanced by the fact that some of these cells show in ragged outline skeletons, as it were, of once larger chromatic masses, and that these cells are prone to show more or less advanced nuclear changes and irregularity of dendritic processes. Degenerative progress is shown, however, in these cells, as in the cells already described from other parts of the nervous system of these same rabbits, by changes in the nucleus and in the outlines of the cell body and processes.

While in most cells the nuclear membrane appears with increased distinctness, its outline is irregular, this irregularity varying from a slight depression or projection at one point to marked crenation of the entire nucleus. There is usually more or less diffuse staining of the nucleus, its depth depending largely upon the amount of shrinkage, being slight in nuclei of normal size, and extreme in those with marked shrivelling and crenation. The condition of the nuclear reticulum varies, its clearness, of course, depending largely upon the extent of the diffuse staining. It is often seen to be quite disintegrated, however, in nuclei which show no general staining. The nucleolus is usually ragged and irregular, sometimes broken up into several pieces.

The outline of the cell body shows, in most cases, some irregularity; it may be merely a waviness of contour, or less frequently a decided shrinkage of the cell body around the nucleus. Between

these extremes are all gradations. There is usually a fine line of chromatic granules around the periphery, emphasizing the raggedness of the cell contour. The same irregularity extends out into the dendrites, often more marked there than in the cell body proper. The varicosities are often quite large, and between them the process may be reduced to a mere thread. A finely granular chromatic substance is abundant in the varicosities. The diameter of a process through one of these varicosities is usually not greater than that of the normal dendrite, while between them the diameter is greatly reduced. It would thus seem that the varicose condition is due mainly to shrinkage between, rather than swelling at, the points of the varicosity, and that the varicosities themselves depend upon clumps of basophile granules. Plate XXXVIII, Fig. 25, a, shows one of these more changed cells, and in b of the same figure is seen an atrophied dendrite containing basophile granules. The staining is by methylene blue alone.

Mitral cells of the olfactory lobe. The description already given for the mitral cells in Rabbits Nos. 5 and 6, applies equally to those in Rabbits Nos. 7, 8, and 9, as regards the general character of the changes. There has been a further decrease, however, in the number of cells, and more of those remaining show the more intense grades of change. No normal cells are present, and few show any large chromatic masses. Fine granules strung out in ragged wavy lines represent the remnants of the Nissl bodies. Sometimes in rough outline are seen the skeletons of the larger Nissl bodies, described in other cells. There is little if any further loss in chromatic substance. In many even of the most shrunken cells the main dendrites appear of nearly normal diameter, though extremely wavy. In these are seen the fine, rough, broken lines of blue granules lying upon the strands of an only slightly disintegrated acidophile reticulum. Other more shrunken dendrites are varicose, devoid of reticulum, stain diffusely dark red, and show an arrangement of chromatic substance like that described in the cortical cells similarly affected. Raggedness of outline of cell body and of nucleus, with shrinkage of both, is the rule. No more striking picture is seen in any part of the central nervous system of these rabbits, than that presented by this

row of mitral cells. In the normal olfactory lobe these cells present a very uniform appearance and even distribution along the edges of the granular layer; the bulk of their chromatin is in large blocks, is very dense and stains intensely. When one examines, even with a low magnification, this line of mitral cells in any of the later rabbits of the series, the changes are seen to be extremely pronounced. The long stretches entirely free from nerve cells, the ragged, shrunken bodies of most of those remaining, with their shrivelled and eccentric nuclei and disintegrated chromatic bodies, the many long, irregular, wavy parts of dendrites, scattered along even where there are no cell bodies, present altogether a very striking picture.

Basal ganglia. The large cells here show about the same grade of lesion as do the stichochromes of the ventral horn, the description of the latter applying equally to the former. There are almost no normal cells. Most of the cells are in a condition of well advanced degeneration; in many there is extreme chromatolysis, even more marked than that depicted in Plate XXXVIII, Fig. 22. The types pictured in Plate XXXVI, Fig. 13, are frequent, the same hard wavy lines seen in cell b of this figure being present. Nuclear changes are identical with those already described in the ventral horn cells. Most of the small cells show almost complete chromatolysis, with distortion of the cell bodies, loss of processes and more or less complete disintegration.

I desire to express my thanks to Dr. Follen Cabot and to Mr. James P. Atkinson of the Research Laboratory of the Department of Health of the City of New York, for material placed at my disposal and for their courtesy in the inoculation of a series of rabbits with the virus of rabies.

BIBLIOGRAPHY FOR THE NORMAL NERVE CELLS.

1. Nissl.—Ueber die Untersuchungsmethoden der Grosshirnrinde. *Tagebl. d. 58. Versamml. deutscher Naturf. u. Aerzte in Strassburg* (1885), p. 506.
2. Key and Retzius.—Studien in der Anatomie des Nervensystems und des Bindegewebes. Stockholm, 1876.
3. Arndt, R.—Untersuchungen über die Ganglienkörper des Nervus sympathicus. *Arch. f. mikr. Anat.*, 1874, x, p. 208.

4. Nissl.—Ueber die Nomenklatur in der Nervenzellenanatomie und ihre nächsten Ziele. *Neurolog. Centralbl.*, 1895, xiv, pp. 66; 104.
5. von Lenhossék.—Der feinere Bau des Nervensystems im Lichte neuester Forschungen. 2. Aufl. Berlin, 1895.
6. Held.—Beiträge zur Structur der Nervenzellen und ihrer Fortsätze. *Arch. f. Anat. u. Entwcklngsgesch.*, 1895, p. 396.
7. de Quervain.—Ueber die Veränderungen des Centralnervensystems bei experimenteller Kachexia thyreopriva der Thiere. Virchow's *Archiv*, 1893, cxxxiii, p. 481.
8. Halliburton.—A Text Book of Chemical Physiology and Pathology. London, 1891.
9. Macallum.—Some points in the micro-chemistry of nerve cells. *British Med. Journ.*, 1898, ii, p. 778.
10. Remak.—Neurologische Erläuterungen. *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1844, p. 463.
11. Max Schultze.—Stricker's Handb. d. Lehre v. d. Geweben, Bd. i, p. 128. Leipzig, 1871.
12. Kronthal.—*Neurolog. Centralbl.*, 1890, ix, p. 40, and 1895, xiv, p. 795.
13. A. S. Dogiel.—Zur Frage üb. d. Bau d. Nervenzellen, etc. *Arch. f. mikr. Anat.*, 1893, xli, p. 62; Die Structur d. Nervenzellen d. Retina. Ibid., 1895, xlvi, p. 394; Der Bau d. Spinalganglien. *Anat. Anzeiger*, 1896, xii, p. 140.
14. Flemming.—Bibliography reviewed up to date in Beiträge zur Anat. u. Embryol., als Festgabe für J. Henle, p. 12. Bonn, 1882.
Ueber den Bau der Spinalganglienzellen bei Säugethieren, etc. *Arch. f. mikr. Anat.*, 1895, xlvi, p. 379. Die Structur der Spinalganglienzellen bei Säugethieren. *Arch. f. Psychiat. u. Nervenkr.*, 1897, xxix, p. 969.
15. Müller, E.—Untersuchungen über den Bau der Spinalganglien. *Nord. med. Arkiv*, 1891, n. F., i, p. 1.
16. Bethe.—Ueber die Primitivfibrillen in den Ganglienzellen vom Menschen und anderen Wirbelthieren. *Morphol. Arb.*, 1898, viii, p. 95.
17. Becker.—XX. Wanderversammlung der südwestd. Neurologen und Irrenärzte in Baden-Baden (1895). *Arch. f. Psychiat.*, 1895, xxvii, p. 953.
18. Apáthy.—Das leitende Element des Nervensystems und seine topographischen Beziehungen zu den Zellen. *Mittheil. a. d. zoolog. Station zu Neapel*, 1897, xii, p. 495.

19. Ramon y Cajal.—Estructura del protoplasma nervioso. *Revista trimestral micrográfica.* Madrid, 1896, i, p. 1.
20. Held.—Beiträge zur Structur der Nervenzellen und ihrer Fortsätze. *Arch. f. Anat. u. Entwcklngsgesch.*, 1897, Suppl.-Bd., p. 273.
21. Van Gehuchten.—*Neurolog. Centralbl.*, 1897.
22. Van Gieson. Discussion on article by Ewing, "A preliminary communication on studies on ganglion cells." *Med. Record* (New York), 1898, liii, p. 525.
23. Ewing.—Studies on Ganglion Cells. *Arch. of Neurol. and Psycho-Path.*, 1898, i, p. 263.
24. Rosin.—Ueber eine neue Färbungsmethode des gesammten Nervensystems nebst Bemerkungen über Ganglienzellen und Gliazellen. *Neurolog. Centralbl.*, 1893, xii, p. 803.
25. Rehm.—*Münch. med. Wochenschr.*, 1892, p. 217.
26. Ewing.—Op. cit., p. 30.
27. Ranvier.—Traité technique d'histologie. 2. éd. Paris, 1889.
28. Boll.—Studi sulle immagini microscopiche della fibra nervosa midollare. *Atti d. r. Accad. d. Lincei. Cl. di sc. fis., mat. e nat.*, Roma, 1876.
29. Schwalbe.—Lehrb. d. Neurologie, p. 302. Erlangen, 1881.
30. Bütschli.—Untersuchungen über mikroskopische Schäume und das Protoplasma. Leipzig, 1892.
31. L. F. Barker.—The Nervous System and its Constituent Neurones. New York, 1899.

BIBLIOGRAPHY FOR RABIES.

32. Richard Mead.—A Mechanical Account of Poisons. 4. ed., p. 129. London, 1747. (The first edition was published in 1702.)
33. van Swieten. Commentaria. Paris, 1771.
34. Gamaleia.—*Ann. de l'Institut Pasteur*, 1887, i, p. 165.
35. Schaffer.—Ibid., 1889, iii, p. 644.
36. Benedikt.—*Wiener med. Presse*, 1874, xv, p. 617, and Virchow's *Archiv*, 1875, lxiv, p. 557; 1878, lxxii, p. 425.
37. Kolessnikow.—*Centralbl. f. d. med. Wissensch.*, 1875, p. 853, and Virchow's *Archiv*, 1881, lxxxv, p. 445.
38. Coats.—*British Med. Journ.*, 1877, ii, p. 866.
39. Gowers.—*Trans. Path. Soc. London*, 1877, xxviii, p. 10.
40. Babes.—Studien über die Wuthkrankheit. Virchow's *Archiv*, 1887, cx, p. 562.

Sur certains charactères des lésions histologiques de la rage. *Ann. de l'Institut Pasteur*, 1892, vi, p. 209.

Ueber den Einfluss der verschiedenen Infectionen auf die Nervenzellen des Rückenmarks. *Berliner klin. Wochenschr.*, 1898, pp. 6; 36; 56.

Sur les lésions précoces des centres nerveux dans la rage. *Compt. rend. Acad. d. sc.*, 1898, cxxvii, p. 776.

Les nodules rabiques et le diagnostic rapide de la rage. *Presse méd.*, 1900, ii, p. 169.

Le diagnostic rapide de la rage par l'examen microscopique du bulbe du chien mordeur. *Ann. de méd. vétér.*, 1900, xlix, p. 252.

41. Gianturco.—Ricerche istologiche sulla rabbia. *Psichiatria*, 1887, v, p. 299.
42. Golgi.—Ueber die pathologische Histologie der Rabies experimentalis. *Berliner klin. Wochenschr.*, 1894, p. 325.
43. Germano and Capobianco.—Contribution à l'histologie de la rage. *Ann. de l'Institut Pasteur*, 1895, ix, p. 625.
44. van Gehuchten and Nelis.—Les lésions histologiques de la rage chez les animaux et chez l'homme. *Bull. Acad. roy. de méd. de Belg.*, 1900, 4. s., xiv, p. 31.

Nelis. Étude sur l'anatomie et la physiologie pathologiques de la rage. *Arch. de biologie*, 1900, xvi, p. 601.

van Gehuchten. La rage. *Ann. de l'Inst. chir. de Bruxelles*, 1900, vii, p. 105.

van Gehuchten and Nelis. Diagnostic histologique de la rage. *Ann. de méd. vétér.*, 1900, xlix, p. 243.

van Gehuchten. A propos du diagnostic histologique de la rage des rues. *Semaine méd.*, 1900, p. 169. Idem. *Journ. de neurologie*, 1900, Nos. 19 and 20, and *Bull. Acad. roy. de méd. de Belg.*, 1900, 4. s., xiv, p. 389.

45. Crocq.—Les lésions anatomo-pathologiques de la rage sont-elles spécifiques? *Journ. de neurologie*, 1900, v, p. 241.
46. Pollaillon and Nepveu.—Un cas de rage. *Comptes rend. Soc. de biol.*, 1872, p. 132.
47. Hébrant.—Sur les lésions de la rage chez le chien et sur le diagnostic post-mortem de cette affection. *Ann. de méd. vétér.*, 1900, xlix, p. 76; also, ibid, 1900, xlix, p. 302, and p. 569.
48. Nocard.—Sur le diagnostic post-mortem de la rage du chien. *Bull. de l'Acad. de méd.* (Paris), 1900, No. 16, p. 476.
49. Cuillé and Vallée. *Revue vétér.*, June, 1900.
50. Ravenel and McCarthy.—The rapid diagnosis of rabies. *University Medical Magazine* (Philadelphia), Jan., 1901, xiii, p. 766, and *Proc. Path. Soc. Philadelphia*, 1900, n. s., iii, p. 231.

51. Gratia.—Un cas de rage humaine. *Bull. Soc. roy. d. sc. méd. et nat. de Bruxelles,* March 6, 1899; also *Clinique* (Brux.), 1900, xiv, pp. 465; 481; and *Ann. de méd. vétér.,* 1900, xlix, p. 345.

52. Sano.—Lésions anatomo-pathologiques de la rage des rues. *Ann. de la Soc. méd.-chir. d'Anvers,* March and April, 1900.

53. de Buck and de Moor.—*Journ. de neurologie,* March 14, 1900.

54. Spiller.—Remarks on the importance of the so-called specific lesions of rabies. *University Medical Magazine,* Jan., 1901, xiii, p. 776.

DESCRIPTION OF PLATES XXXIV-XXXVIII.

Unless otherwise noted, drawings were made with a Reichert $\frac{1}{12}$ homog. imm., apert. 1.25, from specimens 6μ thick, stained with erythrosin-methylene-blue.

The Normal Nerve Cells.

Plate XXXIV.

Fig. 1. Typical spinal stichochrome from ventral horn of cord of a normal rabbit. The acidophile reticulum is seen in the axone hillock extending out as delicate longitudinal striations into the axis cylinder. In the protoplasmic processes the reticular meshes are elongated.

Fig. 2. Drawing made with a Reichert $\frac{1}{18}$ homog. imm., apert. 1.30, sections 3μ thick, representing portions of ventral-horn stichochromes.

a. A protoplasmic process in longitudinal section, showing elongated meshes of the acidophile reticulum and the longitudinally arranged chromophilic spindles lying upon them.

b. Cross section of a protoplasmic process showing acidophile reticulum and cross-cut chromatic spindles lying on and around the strands of the reticulum.

c. Longitudinal section of an axone, showing striations and their relations to the intra-cellular reticulum.

Fig. 3. Drawings made with a Reichert $\frac{1}{18}$ homog. imm., apert. 1.30, sections 3μ thick, representing portions of ventral-horn stichochromes. Shows relation of basophile granules to acidophile reticulum. Granules are seen to lie upon meshes of reticulum.

Fig. 4. From a spinal ganglion, lumbar region; section 6μ thick. Shows types of cells found in spinal ganglia with the variations in size of cells, in size of nuclei, and in the size and arrangement of the chromophilic granules. In two of the cells (a and a′), one large and one small, the chromophilic granules are arranged as short rods curved slightly so as to present a concavity towards the nucleus and giving a concentric appearance to their arrangement. Another cell (b) shows the fine granular condition of the chromatic substance with an almost even distribution throughout the cell-body, there being a few larger chromatic masses present. The fourth cell (c) shows a rather coarse reticular arrangement of the granules, the reticulum corresponding somewhat to, though being coarser meshed than, the acidophile reticulum.

Fig. 5. Typical Purkinje cells from the rabbit's cerebellum. In one of the cells, a, the plane of section includes a large part of the main dendrite with primary and secondary bifurcations and gives a clear picture of the dendritic reticulum and the

overlying rods of granules. The nucleus is rather small as compared with the cell-body, and its reticular strands are coarse. In all of the cells drawn the "nuclear cap" is distinct, as is also a tendency, not nearly so marked as in the cells of the spinal ganglia, to a concentric arrangement of the granular masses.

PLATE XXXV.

Fig. 6. Represents two cells from the mitral layer of the olfactory lobe. The chromophilic bodies are extremely coarse, and there is a well marked nuclear cap. The rods in the dendrites are large. The chromophilic masses take a very dark stain. The acidophile reticulum is of comparatively fine mesh, and does not show well any distinct relation to the chromophilic granules.

Fig. 7. Four cells from the frontal lobe of the cerebrum. One of these (a) is cut at right angles to the direction of the main dendrite, and is from a section tangential to the surface. The others are cut in the axis of the main dendrite and are from transverse sections cut at right angle to the surface of the brain.

Fig. 8. Three cells from the cerebrum. The section was a perpendicular one, and taken at a point midway between the tip of the frontal lobe and the tip of the occipital. The cells average considerably larger and are richer in chromatic substance than in the frontal lobe.

Fig. 9. Five cells from the occipital lobe. These again are smaller than those found in the mid-region, and the chromatic constituent is less.

RABIES.

PLATE XXXVI.

Fig. 10. From ventral-horn cells, lumbar cord, Rabies Rabbit No. 1. Sections 3μ thick; Reichert $\frac{1}{18}$ homog. imm., apert. 1.30. Early changes in the chromophilic bodies and relation of these to the acidophile reticulum.

a. Frayed out appearance of edges of chromophilic bodies without any diminution in staining intensity.

b. Frayed edges with considerable diminution in staining intensity.

c. Same as b, but with holes or vacuoles in the chromophilic bodies. These holes seem to correspond to the meshes of the reticulum.

Fig. 11. Cell of ventral horn, cervical cord, Rabies Rabbit No. 3. One of the cells showing most marked changes found in this rabbit. Extreme eccentricity of the nucleus, which bulges somewhat from the cell-body; loss of any distinct nuclear contour; no nuclear membrane; no intra-nuclear network; nucleolus broken up into three distinct parts; acidophile element of nucleus forming a stellate granular mass around the nucleolus; some diffuse red staining of the rest of the nucleus. Cell processes short, tapering to a point near the body of the cell; some appear like mere reddish threads. Only one of the processes shows any evidence of either reticulum or chromophilic rods; the other processes show an increased intensity of reaction to erythrosin, but no reticulum. In the cell-body the reticulum is largely obscured. Instead of the usual separate chromophilic bodies there is around the nucleus a diffuse dark-blue granular deposit, while towards the periphery the chromatic granules are arranged in a reticulum, the meshes corresponding to some extent to the remains of the acidophile reticulum.

Fig. 12. Two large mitral cells from the olfactory lobe, Rabies Rabbit No. 3.

In a, all the large chromatic masses have disappeared, what is left of the basophile element being scattered throughout the cell as small clumps of granules, in irregular rods, and in one end of the cell as a distinct reticulum. The nucleus shows a partial disintegration of its reticulum and the stellate collection of red granules around the nucleolus.

In b, some large chromophilic bodies still remain, ragged and frayed out at the edges. In some parts of the cell there is a diffuse granular deposit; in other parts there is a distinctly reticular arrangement of the granules, which lie on the strands of the acidophile net work. The nucleus is distorted, as is also the nucleolus. The changes in the nucleo-reticulum are the same as in a.

Fig. 13. Ventral-horn cells from cervical cord, Rabies Rabbit No. 6.

a. Nucleus crenated, nucleolus broken up, and nucleo-reticulum almost completely disintegrated. In the cell-body the basophile element is represented only by a few scattered granules. The body of the cell stains a diffuse and rather dark red. The processes end in points at a short distance from the cell-body, these points taking a darker red stain than any other portion.

b. Shows disintegration of larger chromatic masses; outlines of some still show; wavy rods of chromatic substance which have a very hard appearance and take an intense dark-blue stain; cytoreticulum normal throughout greater portion of cell; vacuoles in several places, where both reticulum and chromatic substance are wanting.

Fig. 14. Two cells from ventral horn, lumbar cord, Rabies Rabbit No. 9, transverse section.

a. Cell-body shrunken to one side of cell space, leaving strands of cell substance stretching across between the cell and the edges of the space, and presenting the appearance of large vacuoles. No very distinct chromophilic bodies, but the basophile substance present in rather large amount, deposited as more or less closely packed granules, in some places making a coarse reticulum. A single process is seen which takes a dark-red stain.

b. Resembles preceding, except that the destruction has gone farther. At one side is seen a short, pointed protoplasmic process, entirely disconnected with the remains of the cell-body.

Fig. 15. Three cells from a cervical posterior root ganglion, Rabies Rabbit No. 9.

a. Cell-body shrunken somewhat from cell space, leaving a net-work appearance between the cell-body and the edges of the space; diffuse dull-pink staining of cell-body with rather large dark-blue granules scattered through it; nucleus distorted; nuclear reticulum broken; one stellate red mass, about the size of nucleolus, in the nucleus.

b. Cell-body shrunken to dull-pink mass about centre of space leaving strands, and an irregularly shaped band of cell substance crossing between the central mass and edges of space.

c. Cell fills space excepting at one side where there is a large vacuole; nucleus irregular and takes a homogeneous dark-red stain; reticular arrangement of chromatic substance near periphery of cell, and considerable diffuse blue granulation.

Plate XXXVII.

Fig. 16. Portion of a cervical spinal ganglion, Rabies Rabbit No. 6, showing five nerve cells.

a. Only a remnant of nucleus and nucleolus; acidophile reticulum apparently intact; basophile element of cell-body entirely gone.

b. Nucleus nearly normal. Almost no basophile substance in the central portion of the cell; irregular line of chromophilic bodies around the periphery; acidophile reticulum normal.

c. Nucleus normal; cytoreticulum intact; basophile element present as small, dense, intensely dark-blue masses, scattered quite evenly throughout the cell-body; some diffuse granules in one part of the cell.

d. Intra-nuclear network somewhat broken; some chromophilic bodies around the nucleus and at the periphery, acidophile reticulum normal. Near the surface of the cell its strands are covered by the basophile granules.

e. Intra-nuclear network broken; nucleus eccentric; reticulum normal; cell free from basophile element except near nucleus and at extreme periphery.

Fig. 17. Four cells of Purkinje, Rabies Rabbit No. 6.

a. Eccentricity of nucleus; cytoreticulum normal in part of the cell; diffuse red granules in central portion; reticulum broken in other portions of the cell; basophile granules few and situated near the nucleus and at the periphery on the strands of the reticulum.

b. Nucleus distorted and filled with fine blue and coarser reddish granules; nucleolus large and irregular; no basophile element in cell-body; acidophile reticulum shows in some parts of the cell, but is mostly lost in a diffuse red granular deposit.

c. Nuclear outline lost; no nucleo-reticulum or cytoreticulum; diffuse red granular stain of both nucleus and cell-body; small dark-blue granules in nucleus; basophile substance arranged as more or less concentric rods around the nucleus as a centre.

d. Nucleus distorted and shrunken, taking a diffuse dark-red stain, with very fine blue granules scattered through it; nucleolus crenated; no cytoreticulum; no basophile substance; diffuse granular red stain.

Fig. 18. Three of the more changed cells from the deeper layers of cerebral cortex, Rabies Rabbit No. 6. Transverse section through mid-region of brain.

a. Outline of cell-body irregular; few chromophilic bodies around nucleus, at periphery and in dendrites; diffuse blue granules; reticulum shows only at periphery and in dendrites; nucleus irregular and eccentric; nucleo-reticulum broken.

b. Similar to a, excepting the distinctly reticular arrangement of the blue-staining element in parts of the cell.

c. Extreme eccentricity of nucleolus; cytoreticulum very distinct; complete absence of basophile substance.

Fig. 19. Two mitral cells from the olfactory lobe of Rabies Rabbit No. 5, transverse median section.

a. Nucleus eccentric but not much changed; outlines of some of the larger chromophilic bodies well preserved, but bodies themselves ragged and full of holes, giving a fenestrated appearance; reticular arrangement of some of the granules; cytoreticulum normal.

b. Similar to a, except that nucleus is central; shows the arrangement of chromophilic bodies in large dendrite; they are placed along the margins of the process and being somewhat wavy, give the process a wavy appearance.

Fig. 20. Ventral-horn cell, cervical cord, Rabies Rabbit No. 7. Hæmorrhage into pericellular lymph space, pressing cell over to one side.

Fig. 21. Ventral horn-cell, cervical cord, Rabies Rabbit No. 9. Similar to preceding. Shows a vessel from which hæmorrhage has taken place into a pericellular lymph space; cell-body pushed over to one side; irregular, fenestrated, chromophilic bodies packed closely together.

PLATE XXXVIII.

Fig. 22. Ventral-horn cell, cervical cord, Rabies Rabbit No. 1. Cell was situated in the postero-internal part of the horn. Shows an extreme degree of degeneration for this rabbit. Nucleolus irregular in outline and stains irregularly; nucleo-reticulum almost completely lost with the exception of a few thick strands of reddish granules; nuclear outline irregular and indistinct; cell outline ragged and processes gone. Basophile element appears as a fine granular deposit throughout the cell, with here and there a few clumps of granules. Faint acidophile reticulum at the periphery, but throughout the greater portion of the cell the reticulum is either lost or covered up by the diffuse blue granular stain.

Fig. 23. Three of the larger pyramidal cells from the deeper part of the cerebral cortex; transverse section midway between tip of frontal and tip of occipital lobes; Rabies Rabbit No. 5. Disintegration of the nucleoli in two of the cells; stellate arrangement of granules around the nucleolus with breaking up of the nucleo-reticulum; in one of the cells the nucleus is markedly eccentric. Disappearance of the larger chromatic masses with a decidedly reticular arrangement of the remaining granules.

Fig. 24. Cell from motor nucleus of fifth nerve; transverse section; Rabies Rabbit No. 9.

Cell-body appears swollen; smooth in outline; filled with fine granules, some of which take the blue, others the red stain. At one end of the cell a considerable area entirely free from the blue granules and from which a process, probably the axone, arises. No distinct nuclear outline, the area of the nucleus taking a granular-red stain like the axone hill, and in the centre of the area a nucleolus surrounded by a stellate purple area apparently the remains of the shrivelled nucleus.

Fig. 25. Mitral cells, olfactory lobe, Rabies Rabbit No. 9, transverse median section, methylene-blue stain only.

a. Shrunken cell-body; reticular arrangement of blue granules; irregular shrinkage of processes, the chromophilic network showing in the broader parts, remaining chromophilic granules seem to cause nodules on dendrites; nucleus and nucleolus crenated.

b. Reichert homog. imm. $\frac{1}{18}$, apert. 1.30, shows even more distinctly than preceding the arrangement of basophile granules in atrophied dendrite.

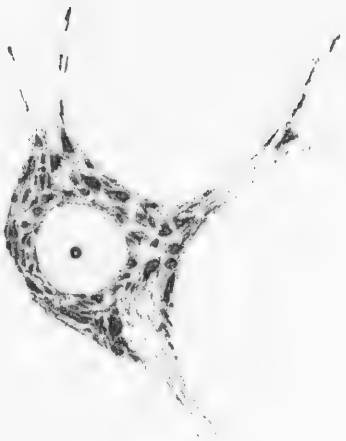

FIG. 1.

FIG. 2.

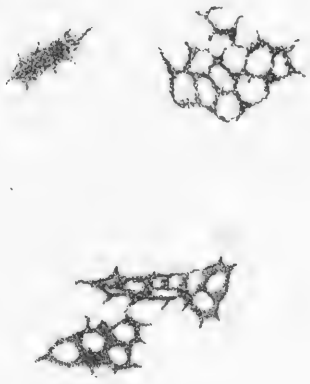

FIG. 3.

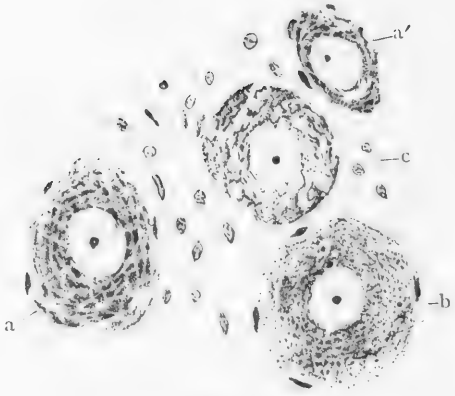

FIG. 4.

FIG. 5.

FIG. 6.

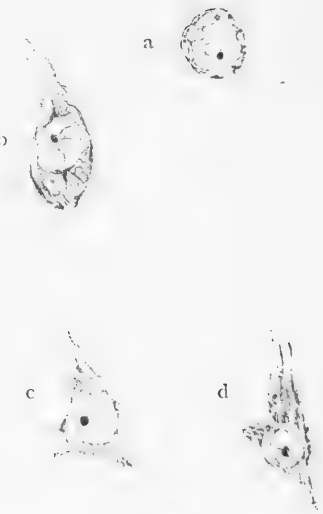

FIG. 7.

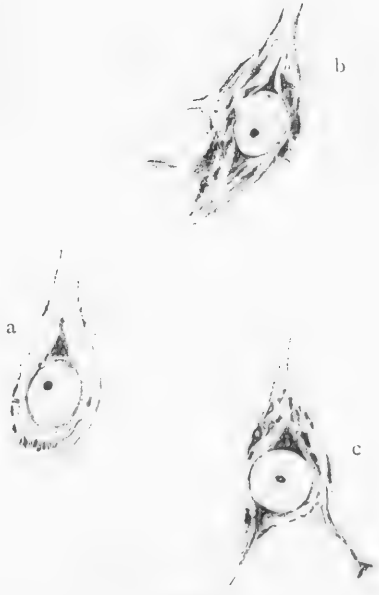

FIG. 8.

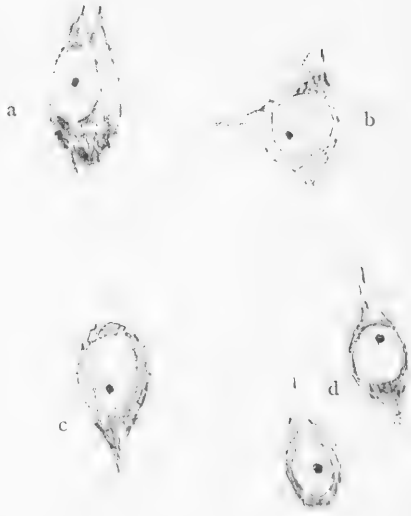

FIG. 9.

FIG. 10.

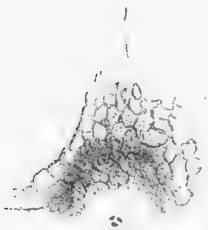

FIG. 11.

FIG. 13.

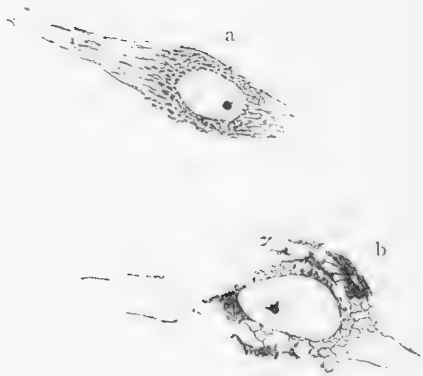

FIG. 12.

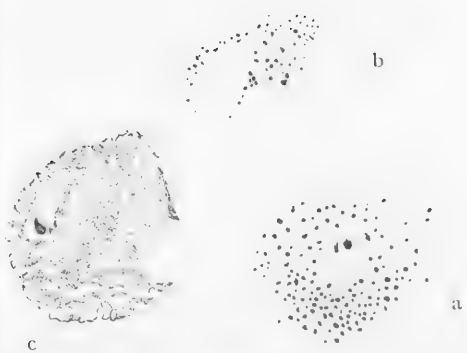

FIG. 15.

FIG. 14.

FIG. 16.

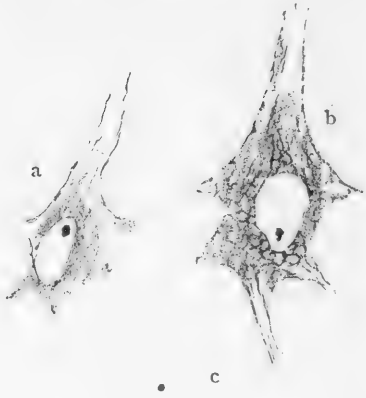

FIG. 18.

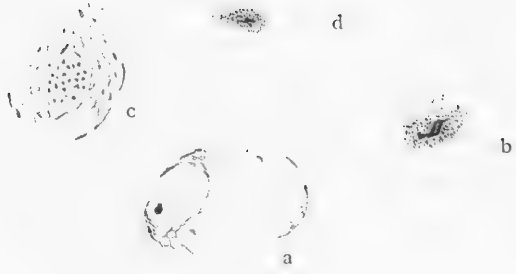

FIG. 17.

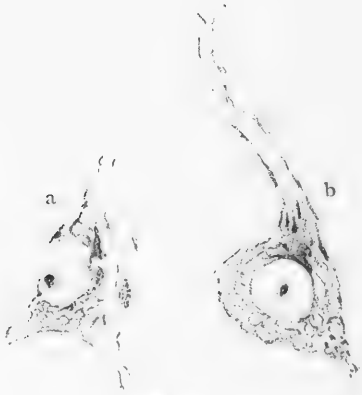

FIG. 19.

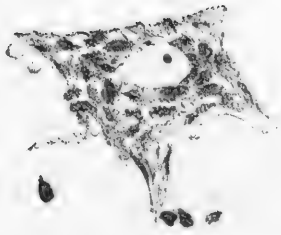

FIG. 20.

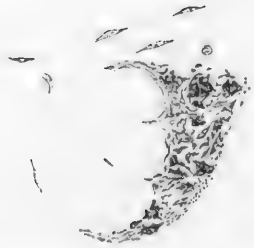

FIG. 21.

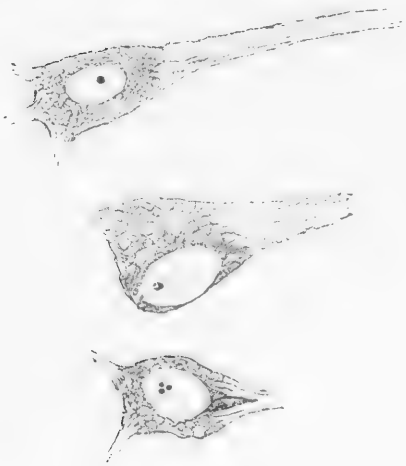

FIG. 23.

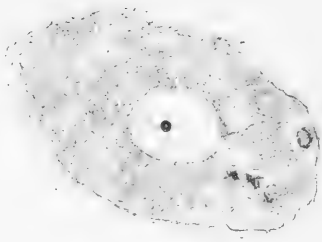

FIG. 22.

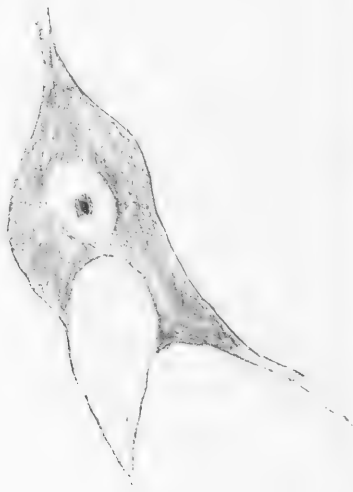

FIG. 24.

FIG. 25.

THE ACID INTOXICATION OF DIABETES IN ITS RELATION TO PROGNOSIS.[1]

By C A. HERTER, M. D.

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While the admirable studies of Stadelmann,[2] Naunyn,[3] Minkowski[4] and Magnus-Levy[5] have firmly established the fact that the peculiar coma so often noted in diabetes is regularly associated with the excretion of large quantities of organic acids, comparatively little attention has been devoted to the study of the excretion of such acids during the period preceding the onset of coma. I desire here to call attention to the appearance of organic acids in the urine previous to the actual development of coma with a view to emphasizing their prognostic significance.

METHODS OF DETECTING THE PRESENCE OF ACIDS IN THE URINE.

In the cases to be reported here, the chief method employed to determine the amount of organic acids excreted has been the process

[1] Read before the Association of American Physicians, May 1, 1901.

[2] Ueber den Einfluss der Alkalien auf den menschlichen Stoffwechsel. Stuttgart, 1890, also *Arch. f. exp. Path. u. Pharm.*, 1883, xvii, p. 419.

[3] *Volkmann's Sammlung klin. Vorträge*, 1889, No. 349–350.

[4] *Arch. f. exp. Path. u. Pharm.*, 1884, xviii, p. 35.

[5] Ibid., 1899, xlii, p. 149.

consisting in the estimation of the acids and bases of the urine. In health the urine contains four chief acids united to five different bases, and these acids and bases very nearly neutralize one another. In diabetes, on the contrary, the total known acids of the urine may fail to neutralize the total quantity of known bases and there is an *apparent* excess of bases. The excess of bases over acids is only apparent, for the urine does not contain free alkali. The apparent excess of bases is in reality united to acids, but these acids are not the *known* acids referred to above; they are organic acids and the apparent excess of bases is proportional to the quantity of organic acid which is being excreted. On the basis of these facts, which were first recognized by Stadelmann, Dr. A. J. Wakeman and I have developed a method[6] of estimating the organic acids of the urine, and this method has been used in the study of our cases of diabetes. It is, of course, to be understood that by this method we do not obtain any clue to the nature of the organic acids, but only a knowledge of their combining power. This knowledge enables us to express the amount of acid present in terms of oxybutyric or any other organic acid, and in references to the quantity of acid excreted in our cases, oxybutyric acid is chosen because it is the preponderating pathological organic acid in cases of diabetes.

The quantity of NH_3 excreted serves roughly to indicate the extent of the excretion of the organic acids. This of course depends on the fact that ammonium is the base chiefly concerned with the removal of organic acids from the body. It is estimated that a daily output of 2 grammes NH_3 corresponds to about 6 grammes of oxybutyric acid, 5 grammes NH_3 to about 20 grammes, and 8 grammes NH_3 to about 36-40 grammes of oxybutyric acid, but I shall show later that considerable amounts of organic acid may exceptionally be excreted without the NH_3 output being above the normal. Nevertheless the method is a valuable one for clinical purposes. We have determined the NH_3 output as part of the process of balancing the acids and bases, but have seldom relied entirely upon it as our evidence of the excretion of organic acids.

In many instances the presence of oxybutyric acid has been ascer-

[6] *The New York University Bulletin of the Medical Sciences*, 1901, i, p. 7.

tained by obtaining crotonic acid from the urine in the well-known manner.[7] Comparatively little attention has been paid to diacetic acid on account of its instability.

THE EXCRETION OF ORGANIC ACIDS SHORTLY BEFORE OR DURING COMA.

The urines from several diabetic patients in a state of coma have come under our observation.

Case I. In the first patient, a young girl in the care of Dr. E. G. Janeway, the bases were so much in excess of the acids, in a separate sample of 340 cc. of urine, that the urine was estimated to contain 27.17 gm. of oxybutyric acid in 24 hours.

Case II. In the second case, a woman brought comatose into the City Hospital, without a history, the excess of bases indicated the excretion of more than 10.8 gm. of this acid in the last 24 hours. In the first case the N of NH_3 was 20.88 per cent of the total N; in the second it was 16.73 per cent of the total N.[8] The second patient came to consciousness after an infusion of sodium bicarbonate, but soon lapsed again into coma. Autopsy showed no lesions which would explain coma except an atrophic pancreas, and the case was regarded as one of diabetic acid intoxication.

Case III. A third case of diabetic coma from which the urine was obtained was in the surgical service of Dr. Markoe in Bellevue Hospital. The small specimen of urine drawn from the bladder after the onset of coma contained 13.5 per cent of N of NH_3, and the excess of bases indicated an amount of organic acid equal to 9 gm. of oxybutyric acid calculated for 24 hours, assuming an excretion of 10 gm. of N in this period. This is doubtless an underestimate of the quantity of acid.

Besides these three cases of diabetic coma, I have had under observation four cases in which coma appeared several weeks after the recognition of large quantities of organic acids in the urine.

[7] 200 cc. of urine from which the sugar has been removed by fermentation with yeast are rendered alkaline with sodium carbonate, and evaporated to the consistence of a syrup. To this syrup, in a flask, 15 or 20 cc. of concentrated H_2SO_4 are added; the mixture is now subjected to distillation, the distillation being continued as long as possible. The benzoic acid in the distillate is partially removed by filtering cold. The filtrate is now shaken with about 50 cc. of sulphuric ether. On evaporation of the ether crotonic acid separates out in characteristic crystals which melt between 69°–71° C.

[8] The following percentages of N of NH_3 were obtained from the urines of different normal persons living on mixed diet; 5.39, 2.87, 3.94, 4.17, 5.22, 3.25, 4.78, 3.66, 3.45, 2.87.

Case IV. One of these was a patient of Dr. J. S. Ely, of New Haven. On Feb. 26, 1901, the urine for the 24 hours contained organic acids equal to 25.96 gm. of oxybutyric acid, and 20 per cent of the N was present as N of NH_3. On March 20 (nearly 4 weeks later), the urine contained acids equal to 23.72 gm. of oxybutyric acid, and 21.28 per cent of the N was present as N of NH_3. In the course of a few weeks this patient died in diabetic coma.

Case V. The second observation relates to a man of 30 (a patient of Dr. H. P. Loomis), who is known to have had glycosuria for at least 6 months, and who had lost considerable weight when the urine was studied, Nov. 1, 1900. On this occasion the excess of bases was equivalent to 24.62 gm. of oxybutyric acid, and 18.45 per cent of the N existed as N of NH_3. Two weeks later this patient died in diabetic coma after a short period of drowsiness, from which he was temporarily roused by an intravenous alkaline infusion. All the urines from this patient that were examined yielded large amounts of crotonic acid.

Case VI. Another case to be included in this category is that of a child, aged 4, in whom glycosuria was detected about one month before the time of our first analysis of the urine. This glycosuria followed the excessive use of sugars and starches and was at first thought to be of alimentary origin. A 24 hours' specimen was obtained at a time when the child was on a diet moderately restricted as regards carbohydrates. This urine contained an amount of organic acid equal to 7.77 gm. β-oxybutyric acid—a considerable quantity for a child of 4 years. Crotonic acid was obtained but only in small amount. The N of NH_3 reached 19.57 per cent, and the total NH_3 was 1.46 gm. Ten days after the time of this analysis the child suffered from headache and was given 2 grains of calomel. The sugar in the urine fell greatly when the calomel began to act, and a sample was obtained which showed only .031 per cent of sugar as indicated by titration with Fehling's solution. The urine at the time of the previous examination had contained 4 per cent of glucose (42 gm. in 24 hours). An ammonia determination was made to see if there was a corresponding diminution in the excretion of organic acid. It was found that the NH_3 amounted to 2.51 gm. when calculated for the 24 hours, and the N of NH_3 amounted to 28.11 per cent. These figures correspond to a large quantity of organic acid and the observation is a striking example of the independence of the excretion of sugar and organic acids. This patient died in coma one month after the last observation mentioned. Diacetic acid was always present in the last month, and the acetone output was increased.

Case VII. The last observation in this group relates to a distinguished scientist, 52 years of age, under the care of Dr. Osler, who had had diabetes for at least 10 years before he came under my observation. The percentage of glucose had gradually increased from 0.5 per cent to 4 or 5 per cent. It cannot be said that the patient exercised sufficient care in regard to the use of carbohydrates. In June, 1900, after a winter of hard work and worry, he began to have headaches at night and giddiness and diplopia during the day. He also showed debility and excessive irritability. These symptoms followed an indiscretion in diet. The urine for 24 hours ending the morning of June 14 (during the disturbance) contained sugar 5 per cent (70 gm. in 24 hours), less than 0.4 gm. of organic acid (calculated as oxybutyric), 4.61 per cent of N of NH_3, and only 0.66 gm. of NH_3. No test was made for acetone or diacetic acid.

On Oct. 9, after a summer of rest, from which there resulted a gain in weight and strength, the urine of this patient contained glucose 4.46 per cent (81.17 gm. in 24 hours), N of NH_3 3.62 per cent, and NH_3 0.629 gm. The organic acids of the urine amounted to 1.8 gm. in terms of oxybutyric acid. The increase in these acids was so small as to be considered of little prognostic significance. Nevertheless, the patient was urged to see Dr. Osler in the hope that he might be induced to take better care of himself. During the end of January, Dr. Osler wrote that our diabetic patient had what appeared to be an influenzal infection, with herpes in the distribution of the 1st division of the 5th nerve. After losing much weight and strength, there was a considerable recovery of weight and some recovery in strength, but after a short interval a boil developed on the leg, the patient became restless, vomited repeatedly and soon died in deep coma. Dr. Futcher (to whom I am indebted for notes on the progress of the disease) states that there was a definite acetone odor to the breath and that the respirations became of a suggestive Kussmaul type. The following notes relative to the urine were given me by Dr. Futcher.

Jan. 22, '01. Sp. gr. 1033, sugar 4.2 per cent (polariscope). Amount of urine not stated. Legal's acetone reaction very marked. Typical Gerhardt's reaction for diacetic acid. β-Oxybutyric acid 0.2.

Jan. 25, '01. Sp. gr. 1037, sugar 4.6 per cent. Acetone and diacetic acid reaction marked. β-Oxybutyric acid not tested for.

Jan. 28, '01. Sugar 7.9 per cent. Acetone and diacetic acid reactions just appreciable and that is all.

Feb. 17, '01. Sugar 6.25 per cent. No diacetic acid or acetone reactions present.

The seven cases included in this group of diabetic patients agree in that they all show the presence in the urine of large amounts of organic acids. This was demonstrated in most instances by the method of balancing the acids and bases of the urine and by the less reliable but still satisfactory method of simply determining the NH_3 of the urine. In all the cases where an attempt was made to obtain crotonic acid it was found, and we can regard this as satisfactory evidence of the presence of oxybutyric acid in the urine. These results are in accordance with those obtained by Stadelmann, Naunyn, Minkowski and Magnus-Levy from patients either actually in coma or a few days or weeks before the onset of coma. The excretion of acids is often greater before the period of actual coma, for the urine may be considerably decreased in volume at the time of unconsciousness. This was the case in the fourth observation, which has been elsewhere reported in detail by Dr. Ely.

The relatively short time that may elapse between an apparently stationary condition with little excretion of acid and a state of intoxication ending in coma is well seen in Case VII. Analyses made in June and October, 1900, showed the patient to be passing very little organic acid and to be gaining in weight and strength. Diacetic and oxybutyric acids appeared during an acute infection in January, 1901. During a short time the patient gained weight rapidly and the reaction for diacetic acid could no longer be obtained. Then after a new infection diacetic acid reappeared and coma soon developed. Thus the patient died in diabetic coma only six months after the urine was carrying away very little organic acid.

I know of no observation on the urine of a patient in diabetic coma that has failed to show the presence of large amounts of organic acids. Our own results, taken in conjunction with those of Stadelmann, Naunyn, Magnus-Levy and others, show that in diabetic coma and the antecedent period the N of NH_3 is increased to between 16 and 30 per cent of the total N. In one of our cases a small portion of urine, representing only a part of the day's excretion, contained only 13.5 per cent of N of NH_3, but it is likely that the average for a day would have been higher. In most instances of coma the N of

NH_3 falls between 18 and 25 per cent of the total N. The actual quantity of acid excreted varies enormously. If the kidneys are inactive the quantity may not exceed 10 or 12 gm. of oxybutyric acid. On the other hand, Magnus-Levy mentions an instance in which the amount was 157 gm.[9]

The increase in the excretion of NH_3 is satisfactorily explained on the hypothesis that this NH_3 is diverted from its usual conversion into urea for the purpose of neutralizing the acid which is constantly leaving the cells to pass into the blood.[10]

CASES OF DIABETES IN WHICH CONSIDERABLE QUANTITIES OF ORGANIC ACIDS WERE BEING EXCRETED AND WHICH HAVE NOT YET DEVELOPED COMA OR IN WHICH THE OUTCOME IS UNKNOWN.

Case VIII. Perhaps the most instructive case in this group is that of a small and emaciated female of 55, in whom glycosuria had existed for about 2 years at the time of her admission to the City Hospital. The urine on Oct. 13, 1900, contained organic acids equal to 13.15 gm. of oxybutyric acid, and 16.34 per cent of the total N existed as N of NH_3. The patient was at this time on a free diet containing a large amount of carbohydrates. The diet was restricted in carbohydrates, and on Nov. 1 the urine contained 8.85 gm. oxybutyric acid, with 11.55 per cent of N as N of NH_3. Crotonic acid was readily obtained. Three weeks later, the diet remaining restricted as regards carbohydrates, the urine contained only 0.98 gm. of organic acid, while the N of NH_3 had fallen to 3.28 per cent. A specimen obtained so recently as March 27, 1901, shows less than 5 gm. of oxybutyric acid in 24 hours, and only 5 per cent of N of NH_3. The recent urines have yielded only small amounts of crotonic acid.

The instructive features of these observations are, first, that the patient has lived nearly seven months in spite of the fact that the urine contained so much organic acid as to call for 16.34 per cent of N of NH_3 to aid in its neutralization, a percentage seldom observed except in patients on the verge of coma; and, second, the marked falling off in the organic acids which followed a moderate restriction in diet and which has continued in spite of a more liberal diet latterly. The

[9] *Arch. f. exp. Path. u. Pharm.*, 1899, xlii, pp. 182–3.

[10] This subject is discussed in my paper in the *Trans. Assoc. American Physicians*, 1900, xv, p. 236.

patient has become very much emaciated and is often somnolent during the day.

Case IX. A similar observation was made on a corpulent man, aged 45, who has had glycosuria for ten years. For many years the rigid exclusion of carbohydrates from the diet was promptly followed by the disappearance of sugar from the urine, but for more than a year the glycosuria has persisted in spite of the severest restriction in diet. On March 18, this patient passed 3700 cc. of urine containing 4.7 per cent of sugar, and the equivalent of 9.26 gm. of oxybutyric acid. After 4 days on a restricted diet, 2380 cc. of urine were passed with 2.75 per cent of sugar and only one gm. of oxybutyric acid. Although the patient continued to live on a somewhat restricted diet, the acids increased to 10.7 gm. of oxybutyric acid in less than one week from the time of the previous observation. The first of the three urines mentioned yielded crotonic acid, and there is no doubt that at least part of the organic acid excreted consisted of oxybutyric acid.

It is of interest that no diacetic acid could be detected in the urine just mentioned, although the examination was promptly made.

An exceptional and highly noteworthy feature of this case is the behavior of the nitrogen of ammonia. The first analysis gave 2.42 per cent of N of NH_3, with a total of 0.82 gm. of NH_3. The second analysis gave 3.27 per cent of N of NH_3, with a total of 1.08 gm. of NH_3. The third analysis gave 4.14 per cent of N of NH_3, with a total of 1.31 gm. of NH_3. These values all fall within the limits of the normal excretion of NH_3. This is noteworthy because the patient was excreting a considerable amount of organic acid, and it is a well-established fact that NH_3 is the principal base to which the organic acids are united when they are excreted. It is clear that in this instance the organic acid must have been united to some other base than NH_3, and there is reason to think that the base is chiefly potassium.[11]

The excretion of the NH_3, the Na and the K, during and before and after the period of restricted diet, is indicated in the following table.

[11] This observation is not entirely conclusive as to whether the base removed by the organic acid was chiefly K or Na, for in our method of balancing the acids and bases of the urine these bases are not determined directly. In this method we assume that the Na of the urine corresponds closely to the Ca excreted and the K is obtained by difference, on the basis of this assumption. The results must therefore be regarded as only approximating the truth.

TABLE I.

Excretion of NH_3, Na, and K in Case IX. (24 hour periods.)

Date.	Diet.	Excretion of organic acid in terms of β-oxybutyric acid.	Excretion of NH_3.		Excretion of Na_2O in terms of Na.	Excretion of K_2O in terms of Na.	Excretion of Sugar.	
			Total.	Per cent. N of NH_3			Total.	Per cent.
Mch. 18, 1901.	Carbohydrates moderately restricted.	9.26 gm.	0.82 gm.	2.42	6.69 gm.	5.10 gm.	174. gm.	4.7
Mch. 26, 1901.	Carbohydrates excluded during 4 days.	1.0	1.08	3.27	7.08	3.32	65.45	2.75
Apr. 1, 1901.	Carbohydrates moderately restricted.	10.7	1.31	4.14	4.98	4.82	101.2	3.68

The urine on which the first observation was made shows the excretion of K_2O, in terms of Na, to be nearly as great as the excretion of Na_2O in terms of Na. These are not normal conditions, since when a normal individual is eating plentifully, as in this instance, the Na_2O is only about one-half as abundant as the K_2O. In the second observation the K_2O has fallen to less than one-half the Na_2O, with very little change in the total Na_2O. This fall in K_2O corresponds to the period of strict diet and small excretion of organic acids, that is to the period in which there is no unusual demand for bases to neutralize acid. In the third observation the organic acids have again increased to considerable proportions and the K_2O equals the Na_2O. We are led to the conclusion that it is chiefly the K which is concerned with the removal of the organic acid.[12] If we had depended in this case on the study of the excretion of NH_3 to determine whether there was any pathological excretion of organic acids we should have reached the conclusion that such acids were not being excreted. Probably the true condition could only have been shown by balancing the acids and bases as was here done, since diacetic acid was not detected and the yield of crotonic acid was very small, and in one instance even questionable.

The patient to whom reference has just been made is slowly losing weight, but is still a well-nourished and vigorous individual, capable of doing exacting physical labor. The fact that the exclusion of carbohydrates no longer suffices to stop the glycosuria indicates a loss of assimilative capacity as compared with the conditions one year ago.

[12] See preceding footnote.

The clinical indications are that this patient is likely to do well for many months in spite of a considerable excretion of organic acids at times.

Case X. The next case in this category is that of a middle-aged man, who came under the observation of Dr. E. K. Dunham. He first noticed the excessive amount of his urine about April 7, 1900. Between April 1 and May 1, 1900, that is in one month, he had lost 50 pounds, but still weighed 147 pounds. He suffered from great thirst, anorexia, and great debility, but was able to bring a large jug of his urine from Englewood, N. J., to the Carnegie Laboratory, carrying it part of the way. The urine for 21 hours amounted to 11,500 cc., and contained organic acids equal to 30.02 gm. of β-oxybutyric acid in 24 hours. The N of NH_3 equalled 17.55 per cent, and the total N of NH_3 was 3.32 gm. The ferric chloride reaction for diacetic acid was strong. In the 24 hours' urine the sugar reached the enormous amount of 556.3 gm. The amount of oxybutyric acid excreted makes it probable that this man was not far from a fatal termination in coma, and it is most unfortunate that the subsequent history of the patient is unknown.

Case XI. A somewhat similar but less acute case came under the observation of Dr. G. A. Spalding at St. Luke's Hospital. A man, aged 28, lost 93 pounds in three months, and for six weeks previous to the analysis passed large amounts of urine. At the time of the examination the chief complaints were debility and great thirst. The urine for a portion of the 24 hours contained 6.01 gm. of oxybutyric acid. The quantity for 24 hours was probably two or three times this amount, judging from the N excretion. The N of NH_3 reached 9.7 per cent of the total N. Crotonic acid was easily obtained from the urine. The patient continued to lose weight and strength, but the ultimate outcome is not known.

Case XII. The last observation to be included in this category relates to a male patient of Dr. C. C. Ransom, 75 years of age, who has had persistent glycosuria for several years. The general health is good and the patient is said to be without symptoms. On Nov. 4 the urine contained an amount of acid equal to 6.9 gm. of oxybutyric acid, with 7.08 per cent of N of NH_3 and 0.98 gm. of NH_3. The urine contained 1.17 per cent of sugar, there being 22.81 gm. in 24 hours. The patient has been in excellent health since this observation was made, and has been on a diet moderately restricted in carbohydrates. On Feb. 19, 1901, the urine contained 0.32 per cent sugar and only 2.2 gm. of oxybutyric acid with 3.31 per cent of N of NH_3.

Perhaps the most important fact brought out by the cases in this group is that a diabetic (Case VIII) may live many months after the appearance of a very high percentage of N of NH_3 in the urine. Sandmeyer's reports on the cases studied and published with Külz show some remarkable illustrations of this fact.[13] One patient (Case 414) died 11 months after the urine contained 3.74 gm. of NH_3 (average of 3 different urines); another (Case 415) lived 15 months after the urine showed 7.23 gm. of NH_3—an enormous quantity. Other treatment than restriction in diet seems to have had little to do with these remarkable instances of prolongation of life. In my Case VIII the patient showed no improvement in symptoms when the alkali treatment was instituted.

CASES OF DIABETES IN WHICH THERE IS EITHER NO PATHOLOGICAL EXCRETION OF ORGANIC ACIDS OR IN WHICH THE EXCRETION IS SMALL.

The cases (Table II) which fall into this group are, with two exceptions (Cases XII and XIV), examples of diabetes of mild form; that is, the patients have none of the obtrusive clinical evidences of diabetes such as thirst, polyuria, and loss of weight and strength. In Case XIV an alcoholic neuritis complicated the condition, and the sudden death was probably connected with the extensive myocardial changes. In cases XVI, XVII, XVIII, XIX and XX the prospects for life appear to be good. Case XIII is one in which the patient is constantly losing weight and is doing badly in other respects. The excretion of organic acids is so small that the unfavorable symptoms cannot be brought into relation with them. Case XV has also progressed unfavorably, with an inclination to somnolence, in spite of the small excretion of acids. In Case XVIII the patient excreted 8.36 gm. of acid in terms of β-oxybutyric acid, but crotonic acid was not positively detected. We cannot, however, feel certain that the urine contained no β-oxybutyric acid because of the imperfections in the methods of obtaining crotonic acid from urine. This case might perhaps have been included in the second group.

[13] E. Külz. Klinische Erfahrungen über Diabetes, Jena, 1899.

TABLE II.

Date.	Clinical notes.	Acid in terms of β-oxybutyric acid.	NH_3		Glucose.		No. of case.
			N of NH_3	Total NH_3	Total.	Per cent.	
				gm.	gm.		
1900. May 8	Female, 35 yrs., glycosuria 6 years, loss of weight, thirst, nervousness, cardiac weakness.	1.85 gm.	4.98%	1.294	199.6	2.9	XIII.
Dec. 5	Same patient, has continued to lose weight and strength.	None	4.24%	0.539	125	4.1	....
Oct. 5	Woman, 50 yrs.; alcoholic neuritis, duration glycosuria unknown. Alcoholic delirium. Died suddenly Nov., 1900. No coma. Interacinar pancreatitis.	1.85 gm.	6.29%	0.58	34.23	3.4	XIV.
Nov. 12	Woman, 75 yrs.; glycosuria 6 years, 3-4% sugar on repeated examination. Moderate loss of weight, debility, slight somnolence.	1.62 gm.	4.08%	....	....	(Average 3.4)	XV.
Nov. 22	Man, 30 yrs.; strong, well nourished, glycosuria for 2 years. Was "cured" at Bellevue Hospital 2 years ago. Drinking hard for 2-3 weeks.	None	5.52%	0.12	208	4.16	XVI.
1901. Feb. 23	Male, 54 yrs.; glycosuria 10 yrs.; from 1-4% glucose. Formerly controlled by exclusion of carbohydrates. Loss of 10 pounds in three weeks owing to restricted diet. Now gaining.	7.05 gm.	3.70%	1.43	....	0.39	XVII.
Mar. 7	Same patient. Carbohydrates excluded from diet for 5 days previous.	1.03 gm.	3.0%	1.0	....	0.06	....
Mar. 28	Same patient on moderately restricted diet, gaining in weight and strength.	1.13 gm.	4.14%	1.3	....	0.88	....
Feb. 21	Male, 61 yrs.; glycosuria 4 yrs.; 1-2%. Disappears when carbohydrates are stopped. General health excellent. Inclination to corpulency, no loss in weight. Carbohydrates restricted.	1.1 gm.	4.12%	0.81	16.18	2.8	XVIII
Apr. 6	Same patient on a diet only moderately restricted in carbohydrates. General condition is excellent.	8.36 gm. No crotonic acid	4.98%	0.91	22.62	1.56	...
Apr. 25	Same patient. Diet almost free from carbohydrates.	0.31 gm.	3.85%	0.67	3.8	0.25	...
May 7	Same patient. 5th day on diet entirely free from carbohydrates, -meat, fat, fish, string beans.	0.84 gm.	4.21%	0.70	....	0.2 or less	....
May 12	Same patient. 10th day on very strict diet.	No pathological acids, i. e., acids in apparent excess of bases	5.08%	0.78	Less than 0.1	Less than 0.1	...
Apr. 6	Female, 60 yrs.; glycosuria for several years, diet only slightly restricted as regards carbohydrates. No symptoms, well nourished.	3.2 gm.	4.75%	0.69	....	0.1	XIX.
May 7	Same patient. Diet unrestricted	2.2 gm.	3.89%	0.552	1.5	0.3	....
May 11	Same patient	No pathological acids, i. e., acids in apparent excess of bases	4.82%	0.585	3.3	0.33	....
May 21	Female, 63 yrs.; general health fair, spare build, rather thin, chronic gout. Glycosuria followed influenza in January, 1901. In April sugar 3.85%, 3.63%, 2.99%, May 19, 1.1	No evidence of pathological acids	3.01%	....	....	Less than 0.13	XX.

THE RELATION BETWEEN THE EXCRETION OF ORGANIC ACIDS AND THE EXCRETION OF GLUCOSE.

An examination of the figures relating to the excretion of acids and of sugar makes it quickly evident that the fluctuations in these constituents of the urine are usually by no means proportional. Thus in Case VIII we find the following conditions:

	Sugar.		Acids.
	Per cent.	Total.	
Oct. 13, 1900	3.57	110.7 gm.	13.15 gm.
Nov. 20, 1900	4.54	147.6 "	0.98

Here we see a marked increase in the sugar excreted with a very striking fall in the quantity of acid.

In Case IX we find somewhat different conditions, as shown in Table I on page 625. In this instance there was a considerable fall in the sugar, but the percentage of decrease in the acids was considerably greater.

Again in Case XVII, although there was a fall, nearly proportional, in sugar and in acids, from Feb. 23 to March 7, this was followed on March 28 by a rise in the excretion of sugar, while the acids remained little changed. It is worthy of note that in each of these three instances the considerable fall in the excretion of acids followed a restriction in carbohydrates of the food. This fact appears to me to be of considerable practical interest. If a patient who is eating carbohydrates liberally has a considerable quantity of organic acids in the urine, there is the chance and even the probability that a restriction in diet will be followed by a considerable fall in this excretion of acid. If, however, a patient passes the same quantity of acid as in the case just mentioned, in spite of the fact that he is getting no carbohydrates, the outlook is likely to be worse than in the former case. In other words, we must test the effect of diet upon the acids just as we test its effect upon the excretion of sugar. Other conditions remaining the same, a quick fall in the acids, in response to diet, is to be considered a favorable indication, while the persistence of a considerable excretion of acid, in spite of diet, is to be considered unfavorable.

It sometimes happens that considerable sugar is excreted when there is little excretion of acid. Thus in Case VII we find on one

occasion an excretion of 70 gm. of sugar with less than 0.4 gm. of acid, and on another occasion 81.17 gm. of sugar with 1.8 gm. of acid. On the other hand, in Case XIX we find only 22.62 gm. of sugar with 8.36 gm. of acid.

Notwithstanding these discrepancies, it is true that large amounts of organic acids generally accompany large amounts of sugar, and conversely that a large excretion of sugar (more than 200 gm. per day) is usually associated with a considerable excretion of organic acids. A small excretion of sugar (25 gm.) is rarely accompanied with considerable amounts of organic acids, but on the other hand, small quantities of acid often go with considerable amounts of sugar (75-200 gm. per day). (See Cases XIII and XVI).

A striking example of the independence of the sugar excretion and the acid excretion has already been referred to in connection with Case VI. Here the excretion of acids, as indicated by the N of NH_3, was very large at a time when the sugar almost disappeared from the urine.

THE NATURE OF THE ORGANIC ACIDS IN THE URINE AND BLOOD OF DIABETES.

Since the independent and nearly simultaneous discovery by Minkowski[14] and by Külz[15] of β-oxybutyric acid in the urine of diabetics, many observations have been made which leave no doubt that when the NH_3 content of the undecomposed urine of diabetes is markedly increased, the presence of β-oxybutyric acid can always be demonstrated. There are two properties of this acid which can be utilized for its detection. One is the formation of crotonic acid under the action of strong sulphuric acid and heat. The other is the levorotary action of the acid.[16] By the use of methods based on these properties, it has been possible to show that most of the organic acid in the urine of patients in coma or in the pre-comatose state is β-oxybutyric acid. Although diacetic acid is probably never absent under these conditions, there is reason to think that it is never present in the large amounts in which oxybutyric acid occurs. Of other acids in

[14] E. Minkowski, loc. cit.

[15] *Zeitschr. f. Biologie*, 1884, xx, p. 165.

[16] The acid can also be extracted directly from the urine.

the urine in diabetes we know little. It seems by no means improbable that other organic acids are sometimes associated with β-oxybutyric and diacetic acids.

As yet there have been few observations on the blood and tissues of diabetics which throw light on the nature of the acid in the blood. It is natural to suppose that as the urine contains so much β-oxybutyric acid in diabetes, the blood must also contain it. The acid has indeed been discovered in the blood by such careful observers as Minkowski and Magnus-Levy. The quantity reported by Minkowski is more than 2.2 parts per mille. Magnus-Levy found the acid in the liver, spleen, muscles, brain and gastric contents. It is of interest that considerable acid was found in the brain. I was unable to obtain crotonic acid either from the liver, blood, or brain of a patient who died in diabetic coma, but do not consider that the presence of β-oxybutyric acid can be excluded in this case.

The discussion of the origin of the organic acids formed in diabetes and their relation to diabetic coma I shall reserve for another publication. I desire to express my indebtedness to Dr. A. J. Wakeman for making most of the analyses on which this paper is founded.

CONCLUSIONS.

It seems desirable to emphasize the following conclusions:

1. A careful balancing of the normal acids and bases of the urine makes it possible not merely to detect the presence of organic acids in the urine, but also to determine approximately the amount of such acids. The method recently described by Herter and Wakeman can be recommended as securing a greater degree of accuracy, for the amount of labor involved, than any other procedure.

2. The determination of the N of NH_3 is a useful procedure for clinical purposes, since it is probably true that a considerable excretion of organic acid (say 15 gm. oxybutyric or more in 24 hours) is always attended by an increased excretion of NH_3. As much organic acid as corresponds to 10 gm. oxybutyric acid may be excreted in 24 hours without causing an increased excretion of NH_3 (Case IX). We cannot therefore rely on the ammonia output to detect moderate quantities of organic acid.

3. Where organic acids are removed in considerable amount without increasing the excretion of NH_3, the acid takes out other alkalies, probably in some instances chiefly K.

4. In cases of diabetic coma the urine always contains a large excess of organic acids and the N of NH_3 is usually increased to 18 to 25 per cent of the total N.

5. Crotonic acid can regularly be obtained from the urines of patients in diabetic coma.

6. The condition of diabetic coma is preceded by a period of days, weeks or months, in which there is a large excretion of β-oxybutyric acid (20 gm. or more in 24 hours), and in which the N of NH_3 is largely increased.

7. Patients whose urines show or have shown a large excretion of organic acids are in danger of developing diabetic coma, but the N of NH_3 may temporarily rise as high as 16 per cent and yet coma may be delayed for more than 7 months (Case VII). The persistent excretion of more than 25 gm. of β oxybutyric acid indicates impending coma.

8. A patient passing 30 gm. of β-oxybutyric acid in 24 hours may still have enough energy and strength to be about all day and perform considerable muscular work (Case X).

9. A patient who has been excreting very little organic acid and has gained weight may within a few months show the presence of considerable quantities of organic acid, and die in typical diabetic coma (Case VII).

10. When the urine contains little or no organic acid there is no immediate prospect of diabetic coma, but patients with such urine are probably liable to most of the other dangers that threaten diabetic patients. The relation between the degree of acid intoxication and the susceptibility to infection seems worthy of special experimental study.

11. Where the urine regularly contains more than 200 gm. of sugar per day there is usually considerable organic acid in the urine and large amounts of acid, indicative of coma, are invariably accompanied by considerable or great glycosuria.

12. Sometimes there is much sugar and little or no acid in the urine, and sometimes there is considerable acid and little sugar. These facts render it desirable to examine the urine of diabetic patients at least once a month with reference to the amount of acid excreted, for the element of acid intoxication must be clearly separated from the element of glycosuria in our study of the progress of a case. In other words, we must recognize the acid intoxication as an important and sometimes as a dominant factor in the prognosis, and this element should be regarded even in those cases of diabetes which have the clinical indications of a mild type of the disease. We may thus hope to prolong life in many instances by taking precautions, as to diet and out-of-door life, which might not otherwise be deemed necessary.

13. The withdrawal of carbohydrate food frequently leads to a considerable reduction in the quantity of organic acids excreted. The reason for this is not yet clear and the phenomenon deserves careful study.

NOTES UPON THE AGGLUTINATIONS OBTAINED BY INTRAPERITONEAL INSERTION OF CELLOIDIN CAPSULES CONTAINING BACILLI AND UPON A MODE OF PREPARING SUCH CAPSULES.

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While carrying out some studies upon agglutinations by the usual method of injecting bacilli, living or dead, into the animal tissues, I was led to try a method of enclosing the bacilli in capsules, which were inserted into the abdominal cavity of the animal, and I found that by this method also the serum gained agglutinative power. The extent and the other characters of such agglutinations will be dealt with in later paragraphs, but before recording my results, it is well, I think, that a few words should be said with regard to the preparation of the capsules. For although Nocard and Roux's[1] work on the micro-organism of pleuro-pneumonia of cattle, and Nocard's[2] experiments on the transformation of human into avian tubercle bacilli, by placing them in sealed capsules within the fowl's peritoneal cavity, have brought increased attention to this method of growing bacteria within the body, free from the intervention of the body cells, there does not exist, to my knowledge, in bacteriological literature, any detailed account of a satisfactory method of preparation of the same, and if I mistake not, the difficulty in making capsules which will not rupture nor leak, has been found so considerable that the method has by many been taken up only to be abandoned.

The idea of using collodion or celloidin capsules is, bacteriologically speaking, of comparative antiquity; quite early in the nineties, a somewhat primitive capsule was employed in the Pasteur Institute by

[1] *Ann. de l'Institut Pasteur*, 1898, xii, p. 270.
[2] Ibid., 1898, xii, p. 561.

Metchnikoff and others. This was formed by taking a glass rod or pencil, dipping it into collodion until the desired thickness of coating was obtained; the capsule was then stripped off the rod and the culture inserted, the mouth tied, and finally, the interstices at the neck were cemented over with collodion. The procedure was first mentioned in an article by Metchnikoff, Roux and Salimbeni.[3]

The defects of this method are obvious: namely, the amount of manipulation required, the long time the capsule has to be kept exposed to contamination while the mouth is being sealed up and the liability for the new coat of collodion at the neck not to adhere thoroughly to the previously dried material. Gradually, it would seem, the method of making these capsules was improved in Paris, though I can find no clear statement of how the capsules were made by Metchnikoff, Roux, and Nocard in their later work.

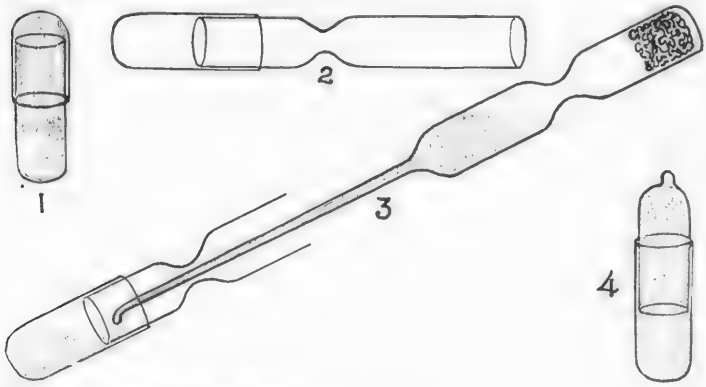
A distinct step in advance was made upon this side of the Atlantic some two years back, so far as I have been able to trace, by Dr. Prudden and others in the Laboratory of the College of Physicians and Surgeons of New York. It consists in employing the gelatin capsules now obtainable at any druggist's, and used for the administration of unpleasant drugs. These capsules are taken as a framework and coated with celloidin; next, the gelatin is dissolved out in a sterile test tube, the cap being then luted upon the body of the capsule by means of painting with thin celloidin. We obtained a knowledge of this method from Drs. Trudeau and Baldwin at the Saranac Lake Laboratory, and, if we are not mistaken, yet further advance was there made by replacing the gelatin cap with a length of glass tubing to which the body of the capsule was luted, the tube being sealed in the flame after filling the capsule. At Saranac Lake they employ a bulb-shaped capsule, and from what I learn from Dr. Baldwin, some little difficulty has been experienced owing to the tendency of capsules so made to undergo rupture within the body and thus set up a general infection.

The method described below has proved so simple and at the same time so successful that a detailed account of it may furnish means to

[3] Toxine et antitoxine cholérique. *Ann. de l'Institut Pasteur*, 1896, x, p. 257.

others of adopting this manner of passing bacteria through the animal body without coming into direct contact with the tissues. The early methods have been touched upon lest I be thought to claim in the slightest degree the credit due to originality.

Celloidin is especially adapted to this work, as it prevents the escape of the organisms while it allows osmosis of the fluids to go on freely. Enclosed in the celloidin capsule the bacilli lie exposed to the body fluids, their soluble products have free egress to the tissues, and, should observations upon the bacilli themselves be desired, the capsule can be removed with a certain knowledge that it contains the form that was originally introduced.



1. Gelatine capsule, natural size.
2. Diagram of glass tube with adherent capsule.
3. Method of filling the capsule.
4. Capsule filled and sealed ready for introduction into the peritoneal cavity; natural size.

A piece of glass tubing, 1 cm. in external diameter and 6 to 8 cm. long, is taken and a narrow and rather abrupt neck is drawn upon it about 3 cm. from one end. It is well, as a matter of precaution, to round off the edges of this end in the flame, lest any sharp edge should later, on manipulation or movement of the capsule within the body, cut through the celloidin. Then over this end, after heating it slightly, the body of a gelatin capsule is fitted, the top being discarded; the hot glass melts the gelatin and there is immediate adhesion. The accompanying diagram 1 gives the exact shape and

size of this capsule with its top. The advantage of this form over the previously mentioned globular forms with neck is that in the latter there is danger of rupture where the neck joins the body, and this danger is done away with here. The glass tubing should pass without difficulty into the capsule (2 in the figure), thereby providing to some extent against the slight shrinkage which may occur during sterilization. The capsule is now repeatedly dipped in thin celloidin, care being taken to dip well beyond the upper edge of the capsule, so that the celloidin adheres directly to the glass; between each fresh coat the capsule is allowed to dry, the dipping being continued until the layer of celloidin is judged to be sufficiently thick.

It is now necessary to melt out the gelatin. I used to accomplish this by first pouring some water into the capsule and then placing the whole in a sterile test tube, with the open end of the glass tube downwards, and heating it in the autoclave for 20 minutes. Lest the melted gelatin refuse to run down the sides of the capsule, a thin wire or fine broom straw may be inserted through the neck of the tube. By this method I obtained a thoroughly practicable capsule and could keep it under sterile conditions without difficulty until it was needed for use. The capsules did, however, exhibit some tendency to shrink, and as shown to me by Dr. C. H. Higgins (who in this laboratory has employed the capsules in his studies upon the tuberculosis of cattle), this shrinkage may be prevented. The modification is as follows:

The capsules are filled with water and placed in test tubes themselves half filled with water, and these are then heated in the autoclave or steam sterilizer. Capsules so treated retain their form admirably, and after emptying out the melted gelatin and half filling with water they may be sterilized and kept, with the open end upwards, in a sterilized test tube containing either water or broth until they are needed.

Into the capsule thus prepared a culture is inserted by means of a fine Pasteur pipette, which is sufficiently small for the capillary tube of the same to pass through the narrow neck (3 in figure). Care must be taken that the inside of the neck is not wetted by the culture,

for if it should be the glass will probably crack during the sealing of the capsule. This step is accomplished by removing the capsule from the test tube by means of a pair of sterile forceps and fusing the narrow neck of the tube rapidly in the blow-pipe flame (4 in figure). The capsule thus sealed must be replaced in the sterile test tube until needed for use. By this wet method of keeping the capsules there is so little danger of contamination that they may immediately be inserted into the peritoneal cavity or elsewhere. When using the dry method, to make sure that the capsule is intact, it is well to keep the sealed capsule in a broth tube for 24 hours, when, if the broth remains clear, the operation may be performed. Nevertheless, one becomes so proficient, that after the first few attempts I was able to use the capsules immediately, and upon subsequent removal from the body found that they were entirely free from any evidence of bacterial growth externally. Until a worker has perfected his technique, it certainly must be laid down that the filled capsule should be preserved in a broth test tube for 24 hours before being placed within the tissues.

The abdomen of the animal is opened antiseptically, the capsule inserted and the wound closed. I have generally found on removal that the capsule has slipped around freely in the cavity; sometimes, however, it is surrounded by adhesions. If it is desired to remove the capsule without killing the animal, the chief practical difficulty lies in finding and recognizing it through the small wound by touch. When found it can be placed in a sterile tube and opened by sterile forceps or scissors.

The agglutinative results, spoken of above, were observed while passing through rabbits some forms of the paracolon group, obtained from Drs. Harvey Cushing, Gwyn and Harris, of the Johns Hopkins Pathological Laboratory and Hospital, Baltimore; their cultural characteristics were tested and found to correspond to Bacillus O (Cushing),[4] B. paracolon (Gwyn),[5] and B. enteritidis (Gärtner). They were inserted in the capsules as 24- or 48-hour bouillon cultures.

The fact elicited in the course of these experiments was that for a

[4] *Bulletin of the Johns Hopkins Hospital*, 1900, xi, p. 156.

[5] Ibid., 1898, ix, p. 54.

few days after the insertion of the capsule, no agglutinative reaction of the serum appeared, even in dilutions of 1 in 10; but on the 10th to the 15th day it began to appear, first in dilutions of 1 in 10 and 1 in 20, gaining in potency daily, until by the 19th to 21st day it would attain a potency of 1 in 1000. Upon the removal of the capsule the agglutinative power decreased day by day, with the same speed with which it had arisen. The agglutinative power of the serum was restricted to the variety of bacillus contained in the capsule, and did not extend to the other, apparently closely related, forms, except in one single instance. If two capsules containing different organisms were put in the same animal, the animal's serum was found to have an agglutinative power over both at the same time, and to about the same degree of potency.

The facts of the experiments are here briefly summarized:

(1) *Rabbit,* with intraperitoneal capsule containing B. paracolon (Gwyn).

Serum: Positive to B. paracolon (Gwyn), on 8th day, 1 in 10, increasing to 1 in 80 on 15th day. Negative to Bacillus O, B. chol. suis, B. icteroides (Sanarelli), B. icteroides (Reed), B. enteritidis (Gärtner), B. morbificans bovis.

(2) *Rabbit,* with intraperitoneal capsule, 1st B. paracolon (Gwyn), 2d (after removal of 1st), B. icteroides (Reed).

Serum: Positive to B. paracolon (Gwyn) and B. icteroides (Reed), and negative to all the others.

(3) *Rabbit,* with capsule of B. enteritidis.

Serum: Positive to B. enteritidis, 1 in 10, on 11th day, increasing to 1 in 1000 on 21st day; capsule removed on 26th day; agglutinative reaction had fallen to 1 in 500 on 31st day. Serum negative throughout to all the other above-named forms.

(4) *Rabbit,* with capsule of Bacillus O.

Serum: Positive to Bacillus O on 13th day; increasing. Positive to B. icteroides (Sanarelli) in 1 in 10; apparently not increasing. Negative throughout to others.

These results will be seen to bear out Cushing's results in his experiments upon Bacillus O, that inter-agglutinations do not necessarily occur between closely related varieties of bacilli.

The above observations do not represent all my studies upon the effects of introducing bacteria in capsules into the peritoneal cavities

of rabbits. They give, however, the results obtained with reference to the agglutinating properties of the blood serum of these animals. I had intended to make the series more complete, but my departure to South Africa arrested my work along these lines at this point. So far as they go, the results obtained by me were so constant and so definite that I feel that I am justified in publishing this note and even in drawing certain conclusions from the results obtained.

Of these results, that which is most obvious is that agglutination, however produced, would appear to be strictly associated with the existence of the bacteria in a living state within the body, for otherwise we cannot explain the fact that removal of the encapsulated bacteria from the peritoneal cavity is followed by the steady disappearance of the agglutinating property of the serum of the animal.

So far as these observations go, they would appear to explain the continued existence of the reaction for months and years in some individuals following upon an attack of typhoid and the rapid disappearance of the reaction in other cases, and this by the continued existence of the bacteria within the tissues in one set of cases and by their complete destruction in the other series. A few years ago this conclusion would have seemed impossible, but now-a-days we are, I think, prepared to accept it, for it is now a familiar experience that typhoid bacteria may be obtained from the gall bladder many months after the patient has apparently wholly recovered from the acute disease, while similarly, long months after the patient has been subjected to the disease we occasionally encounter the bacilli in pure culture in abscesses in the neighborhood of joints and other lesions, observations which prove absolutely that the bacilli may continue for long periods, either lying latent or proliferating very slowly in some one or other region of the body.

In attempting to form a satisfactory theory of agglutination, we possess data which lead us to suppose that the bacteria in culture form certain agglutinins which unite with certain other agglutinins which are the product of the tissues. (The normal serum will, in certain cases, produce in low dilutions an agglutination; but this power is probably not an inherent quality of tissues or serum, but is

caused by the presence of a subinfection by a bacillus nearly related to the bacillus with which the agglutination is made. So rarely does this agglutination by apparently normal serum occur that it may be disregarded.) The fact remains that the tissues, reacted upon by an infection, respond by the production of an agglutinin. By the capsule method we are able to assert that the agglutinin produced by the tissues is not a reaction to the bacillary bodies, nor yet a reaction of the kind called inflammatory, but a reaction to the chemical bacillary products or a combination of the serum with these chemical products.

SUMMARY.

1. Capsules made as described above allow dialysis, when placed in the peritoneal cavity.

2. The normal tissues, unstimulated, do not possess the power of causing agglutination; they do not require to be stimulated by the presence of the bacterial bodies, but will produce their share of the agglutinins when acted upon by the bacillary products.

3. Agglutination follows the insertion, in the peritoneal cavity, of "capsuled" bacilli; it gradually increases in degree, and on the removal of the capsule containing the bacilli, begins to disappear.

4. Varieties of bacilli, related closely in morphology and cultural reactions, do not, as a rule, produce serums which inter-agglutinate.

SOME EXPERIMENTAL DATA ON THE SIGNIFICANCE OF CONCENTRATION AND OF MULTIPLICITY OF AREA IN HYPODERMIC INJECTIONS.[1]

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(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.)

In the theoretical and practical studies of the effects of bacterial toxins and antitoxins upon the animal body, the subcutaneous method of application is extensively employed. Therefore any contribution to the knowledge of the precise working of this method ought to be of interest to bacteriologists. This is my excuse for bringing before this society a subject which in itself is not an integral part of bacteriology.

In a recent communication by v. Czyhlarz and Donath,[2] the assertion was made that the animal tissue is capable of neutralizing or fixing the poisons of strychnine and venom. The fundamental experiment from which this assertion was derived was as follows: The thigh of a guinea-pig was tightly constricted and a fatal dose of strychnine injected into it at a point peripheral to the ligature. When after an hour or two the ligature was removed, the characteristic effect of strychnine did not set in. In a series of experiments by Langmann and myself,[3] it was shown, as I believe conclusively, that this failure is not due to any capacity of the tissue to neutralize poisons, but to the impairment of the capacity of absorption of the constricted leg. One of the experiments which was made in support of our view was as follows: Instead of injecting the minimum effective dose, which is for a guinea-pig of 250 grammes about 1.5 mgr., into one leg, we divided the dose into three parts and injected them in

[1] Read at the meeting of the Society of American Bacteriologists, Dec. 28, 1900, Baltimore.

[2] *Centralbl. f. innere Med.*, 1900, p. 321.

[3] Meltzer and Langmann, *Medical News*, 1900, lxxvii, p. 685.

three extremities which had been constricted prior to the injections. We had here in comparison to the other experiment a greater amount of tissue to a smaller amount of poison, and if the tissues were capable of neutralizing the poison, there should have been surely in this experiment no effect of strychnine visible. Instead, no sooner were the ligatures taken off than violent convulsions broke out. Our explanation was, that there were for the same amount of poison in the three legs more paths of absorption open than in the one constricted leg, hence the positive result.

One observation which I made in the last-mentioned experiment, gave the stimulus to the new series of experiments, the results of which I shall report here briefly. The tetanus, which followed the removal of the ligatures of the three legs, was more violent and set in sooner than in the case in which the sum of the three quantities was injected into one non-ligatured leg. It was now a question, whether the distribution of the same quantity of the solution of strychnine to several areas of the body does not indeed favor a more rapid absorption. The characteristic effect of the poison appears the more quickly and the more pronounced, the more quickly and the more abundantly it is absorbed into the blood and from there into the central nervous system. The absorption from the subcutaneous tissue takes place either through the blood-capillaries or through the lymphatics or in both ways. Now the distribution of the same quantity to several areas brings the poison, it would seem indeed, into contact with a greater number of capillaries and lymphatics, and it would therefore be quite plausible to assume that such a distribution facilitates absorption. On the other hand, the larger the quantity which is injected subcutaneously into one place, the greater is the pressure which it exerts upon the surrounding tissue. And as pressure facilitates filtration, and filtration is at least one of the factors of interstitial absorption, we might suspect that by the distribution one of the factors favoring absorption becomes impaired. Here was a problem and I tried to solve it by experiment. I employed strychnine again, on account of the characteristic reaction and the short time in which a decisive response can be obtained. In the comparison I noted of

course also the length of the interval which elapses between the injection and the appearance of the tetanus and the degree of violence of the latter, but in my positive conclusions I relied chiefly upon the appearance and non-appearance of a tetanus. The following sample experiments illustrate the result:

Rabbit, 1700 grammes, injected into one leg 1 mgr. of strychnine. No effect. Five days later, injected into the same animal 1 mgr. distributed among three extremities. The first tetanus 23 minutes after injection, followed by a few more; animal survived.

It might be suspected in this experiment that some of the strychnine of the first experiment was not yet absorbed and aided in the result of the second injection. The following experiment is free from such a suspicion:

Rabbit, also 1700 grammes, injected 0.9 mgr., distributed among three extremities. Had a number of tonic and clonic convulsions, the first attack 27 minutes after injection; survived. Five days later, the same animal, injected into one leg 1 mgr. of strychnine. No effect.

In this experiment the single injection was even larger than the divided one. Nevertheless convulsions followed the latter, while the former remained ineffective.

My experiments justify the conclusion that in hypodermic injections the distribution of a certain quantity of poison among several places of the body is more effective than the injection of this quantity in a single dose into one place.

Under the influence of the argument that the larger the quantity, the more effective the filtration and the better the absorption, and in the desire to increase the effectiveness of the distribution by increasing the quantity without increasing the dose, I have attempted to employ greater dilutions of the solution of strychnine, but obtained rather puzzling results. I therefore started out to establish, first, the effect of increasing the bulk without changing the dose in single injections. Here my filtration argument received quite a setback. I shall again illustrate the instructive result by a couple of experiments.

Rabbit, 1300 grammes, injected 0.8 mgr. in a dilution of 1:10,000; no effect. Six days later, the same animal, injected 0.6 mgr. in a

dilution of 1:200. Had convulsions after 11 minutes; survived. Five days later, the same animal, injected 0.9 mgr. in a dilution of 1:20,000. No effect. The same animal again, five days later, injected 0.5 mgr. in a 1 per cent solution; violent tetanus after five minutes and dead. 0.5 mgr. in a 1 per cent solution kills the animal in a few minutes, while nearly twice as strong a dose in a dilution of 1:20,000 remained without any effect.

Guinea-pig, 560 grammes, injected 3.4 mgr. in a dilution of 1:20,000; no effect. Ten days later, injected 2.3 mgr. in a 1 per cent solution. Tetanus and death in a few minutes.

These experiments, which gave uniform results, demonstrate unmistakably that the bulk is nothing and the concentration everything; the doses being equal, the more concentrated the injected poison is, the stronger is the result. The meaning is plain: the osmotic pressure is the most important factor in the process of absorption.

The results of these series of experiments are in brief as follows:

The effect of the subcutaneous injection depends to a considerable degree upon the concentration of the injected solutions, and is materially influenced by a greater distribution of the injected quantity over several areas.

OBSERVATIONS ON A CASE OF CYCLIC ALBUMINURIA.

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In recent years considerable attention has been devoted to the transitory or intermittent appearance of small quantities of proteids in the urine. With the establishment of the fact that the urine of healthy individuals may regularly contain appreciable, though small, amounts of albuminous substances,[1] the significance of so-called "physiological" albuminuria has again been called into question. Ordinarily three general types of "physiological" or "functional" albuminuria have been distinguished, viz., the transitory, intermittent and cyclic. These names indicate with sufficient accuracy the variations in proteid excretion which they are intended to differentiate. The term "cyclic" albuminuria has been applied to those conditions in which the proteid is found in the urine only at approximately regular intervals or at definite periods of the day. In how far the distinctions between these various types are actual remains for future investigation to determine; some observers are at present inclined to regard them merely as different expressions or symptoms of the same condition, rather than as the outcome of typical and specific causes.[2] The expression "physiological" albuminuria is an undesirable one, since healthy individuals ordinarily fail to show this symptom. There are, however, many other functions in the animal body where the border-line between the physiological and the pathological occasionally becomes obscure; from the clinical standpoint "physiological" albuminuria may for the present be interpreted as applying to conditions where the excretion of proteid has no abnormal significance. The observations recorded in this paper are intended as a contribution to our knowledge of one type of persistent albuminuria.

The subject of these observations first discovered the presence of

[1] K. A. H. Mörner. *Skandinav. Arch. f. Physiologie*, 1895, vi, p. 332.
[2] C. Flensburg. Ibid., 1893, iv, p. 412.

coagulable proteids in his urine in May, 1899, while a student in the laboratory of physiological chemistry. Since then the excretion of proteid has been studied at various intervals until the present time (February, 1901). During this period no marked change in the quantity eliminated daily has been noted and the general features of the case have remained rather constant. The individual is now 24 years of age, weighs 126 pounds (57.4 kilos), and is 5 feet 7½ inches in height. A very careful physical examination by Professor J. S. Ely failed to indicate any abnormal condition. A recent blood count showing 5,200,000 erythrocytes and 5100 leucocytes per cubic mm. may be taken as typical. Urine samples collected at various times, and particularly during the periods of most pronounced proteid excretion, have been examined after centrifugalization; the collected sediment has not at any time disclosed the presence of unusual structural elements. Furthermore, quantitative determinations of the main urinary constituents have given no evidence of abnormal conditions, as will be seen in the following table:

ANALYSIS OF URINE OF MAY 9, 1900.

Total quantity, 970 cc.; reaction, acid; specific gravity, 1,027

Total Nitrogen	14.39	grammes.
" P_2O_5	2.13	"
" SO_4	2.16	"
Ethereal SO_4	0.16	"
Chlorine as Na Cl	15.68	"
Proteids	0.29	"
Sugar	absent.	

Methods of Observation.—In making the present observations it has been customary to collect the urine at brief intervals (usually hourly or two-hourly periods) during the waking hours. After noting the physical character (specific gravity, volume, reaction, etc.) of each sample, it was filtered and heated for half an hour or more in a boiling water-bath, a few drops of dilute acetic acid being added, when necessary, to facilitate the coagulation of the proteids. The latter were removed by filtration through weighed, ashless papers, washed free from chlorides and dried to constant weight. It was found impracticable to determine the quantity of proteid by analysis of a fraction of the total daily output as was done in the interesting investigation of Sollmann and McComb,[3] since the average daily

[3] *Journal of Experimental Medicine*, 1898, iii, p. 137.

excretion of proteid was considerably smaller, and a satisfactory coagulation was almost impossible in the dilute fluid. This objection did not apply, however, to the hourly examinations; for although proteid was absent from the urine at certain periods of the day, the quantity present in intervening periods was correspondingly larger and thus more easily separated. Furthermore, it seemed especially important to study the time relations in the excretion, hence the frequent collection of the urine.

Average Results.—To demonstrate the typical cycle of proteid excretion in this individual, the results of a few analyses made under ordinary conditions of diet and occupation (laboratory work) are appended. They are selected from a number of observations taken at various times and indicate the character and constancy of the albuminuria.

TABLE I.

URINE EXAMINATION.—HOURLY PERIODS.

DATE.	7.15 A. M.		8 A. M.		9 A. M.		10 A. M.		11 A. M.		12 M.	
	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
5, X, 1900 ..	473	0	35	0	42	17	45	0	130	0	30	10
16, XI, 1900.	185	0	35	20	44	34	70	36	32	15	35	23
25, I, 1901 ..	181	0	35	32	67	17	123	23	52	40	41	32
Averages ...	283	0	35	18	51	23	79	20	71	18	35	22

DATE.	1 P. M.		2 P. M.		3 P. M.		4 P. M.		5 P. M.		6 P. M.	
	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
5, X, 1900 ..	58	0	30	0	47	37	55	0	43	0	69	0
16, XI, 1900.	29	46	35	158	33	137	21	63	28	27	36	18
25, I, 1901..	37	25	55	12	42	15	43	17	40	19	36	26
Averages ...	41	24	40	57	41	63	40	27	37	15	47	15

DATE.	7 P. M.		8 P. M.		9 P. M.		10 P. M.		Day's Totals	
	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
5, X, 1900.....	60	0	23	14	30	29	90	0	1280	107
16, XI, 1900	40	35	45	30	73	0	65	0	806	642
25, I, 1901.....	51	14	70	4	60	13	82	11	1015	298
Averages.....	50	16	46	16	54	13	79	4	1034	349

TABLE I—*Continued.*

URINE EXAMINATION.—TWO-HOURLY PERIODS.

DATE.	7.15 A. M.		8 A. M.		10 A. M.		12 M.		2 P. M.	
	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
16, III, 1900	195	0	50	25	86	20	62	70	87	43
17, III, 1900	164	14	24	20	83	42	45	76	55	54
21, III, 1900	185	1	70	17	70	22	78	28	62	87
7, VI, 1900	80	0	37	14	232	1	105	14	75	33
Averages	156	4	45	19	118	21	73	49	70	54

DATE.	4 P. M.		6 P. M.		8 P. M.		10 P. M.		Day's Totals.	
	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
16, III, 1900	85	79	56	35	65	18	123	6	809	298
17, III, 1900	95	21	95	56	108	33	627	6	1294	321
21, III, 1900	57	86	70	35	86	177	71	22	749	474
7, VI, 1900	60	46	70	40	64	27	60	13	783	177
Averages	74	58	73	42	81	64	220	12	909	318

The average daily excretion of coagulable proteid on these 7 days was 331 mgrs. The hourly variations in the quantity excreted correspond quite closely with those observed by previous investigators for this type of albuminuria.[4] It has been found that while the individual remains in bed, and perhaps during the period immediately after rising, the urine is usually free from coagulable proteid. The excretion then begins, gradually increases in quantity until about noon, when it falls off again; a second but smaller rise occurs later in the afternoon, and towards evening the output is again greatly diminished.

To account for such phenomena various factors have been taken into consideration, such as excessive muscular movements, psychical activity, dietetic influences, and in particular the changes in the posture of the body. There are cases in which each of these influences may apparently be the determining factor. In the present instance an attempt has been made to study the influence of some of

[4] L. Krehl. *Pathologische Physiologie*, p. 463, Leipzig, 1898.

the conditions referred to, and the results of these observations are detailed below.

Influence of Diet.—In their study of a case of cyclic albuminuria, Sollmann and McComb,[5] like many previous observers,[6] failed to detect any changes due to the character of the diet. Our own observations during days in which the food ingested contained a preponderance of either carbohydrate or proteid (meat and eggs), or during fasting, likewise gave no evidence of concomitant variations in the proteid excreted. (Cf. Table II.)

In order to determine still more positively the possible influence of ingestion of food on the excretion of proteid, the following experiment was tried. The subject was awakened from deep sleep at 3 a. m. and ate a rather hearty meal, consisting of bread and butter, milk, chops, marmalade, cakes, etc. The mealtime occupied about 15 minutes, during which the individual was careful not to move from the recumbent position and immediately thereafter he fell asleep again. The urine collected on arising at 7.15 a. m. was entirely free from proteids, corresponding with the experience gained under ordinary conditions. Similar in its bearings on the origin of the excreted proteid is the fact fails to occur regularly during the periods of heightened digestive evident in all the tables that the most pronounced proteid output activity. The total excretion of proteid on the day following this experiment was 355 mgr. in 1168 cc. of urine—figures quite comparable with those obtained on other days.

Influence of Muscular Work.—To ascertain the influence of vigorous muscular exertion, observations were made on days in which strenuous exercise—long walks—was indulged in. No rise in proteid output was observed. Thus, in one experiment recorded in the appended table (Table II, 24, I, 1901), the individual arose at 5 a. m. and took long walks at a brisk pace during various periods of the day, in addition to performing his regular work in the laboratory. Nevertheless the cyclic course of the albuminuria showed no noticeable changes and the day's output of proteid reached a total of only 327 mgr.

[5] Loc. cit.

[6] G. Klemperer. *Zeitschr. f. klin. Med.*, 1887, xii, p. 177.

Influence of Posture.—The influence of the posture of the body on the excretion of proteid in cyclic albuminuria of the Pavy type has been especially studied by K. Osswald.[7] He concludes from observations on three subjects and a study of the records of previous writers that a quiet horizontal posture always diminishes the intensity of the albuminuria; and muscular exercise during the maintenance of this posture fails to incite an excretion of proteid unless an excessive amount of work is done. The upright position, however, is of decided influence, while work done in the sitting posture is without effect. With these observations our own experience in the present instance closely corresponds. Proteid was never present in the urine when the subject arose from bed; but in order to exclude any possible influence of the period of the day aside from personal factors, the individual remained in bed during the daytime and the influence of the horizontal posture was studied when all the other elements were unchanged. The absence of proteids from the urine was repeatedly demonstrated under such conditions and is well illustrated by the protocol of 1, II, 1901 (Table II), where the albuminuria did not begin until the subject arose at 4 p. m.

Nature of the Proteids.—Urine collected during the hours of most pronounced albuminuria showed albumin to be the most conspicuous proteid present; for while we have always obtained a precipitate of proteid (with some urate) when ammonium sulphate was added to saturation, treatment of the urine with saturated ammonium sulphate solution added to half-saturation failed to show even traces of globulin. Nucleo-albumin was not detected even when the urine was acidified after dialysis.[8] Proteoses were also absent.

SUMMARY.

The preceding observations record a new instance of the occurrence of cyclic albuminuria in an otherwise apparently healthy young man. The typical course of the proteid excretion from hour to hour under

[7] *Zeitschr. f. klin. Med.*, 1894, xxvi, p. 117. Appended to this paper will be found an extensive list of references to the literature on cyclic albuminuria.

[8] C. Flensburg. *Skandinav. Arch. f. Physiologie*, 1893, iv, p. 416.

various conditions has been reviewed and its independence of the changes in diet or muscular work pointed out. No relationship between the volume of urine eliminated and the quantity of proteid excreted has been ascertained. The specific effect of the horizontal posture in dispelling the albuminuria is the most interesting feature observed. The attempt to refer this to attendant circulatory changes in the kidneys is, for the present, no more than an interesting speculation.[9]

TABLE II.

URINE EXAMINATION.—EXPERIMENTAL CONDITIONS.

DATE.	REMARKS.	5 A. M.		6 A. M.		7.15 A. M.		8 A. M.	
		Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
4, IV, 1900	Diet largely carbohydrate	..	..	..	..	143	0	31	25
5, IV, 1900	Diet largely carbohydrate	..	..	..	..	111	0	29	26
8, IV, 1900	Subject remained in bed until 5.30 P. M.	..	..	..	..	143	0	62	0
4, XII, 1900	Diet largely proteid.	..	..	..	..	193	0	120	1
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	..	..	..	..	93	0	29	10
23, I, 1901	Additional hearty meal in bed at 3 A. M.	..	..	..	..	112	0	25	21
24, I, 1901	Arose at 5 A. M. Vigorous exercise all day.	180	0	52	24	100	10	177	1
1, II, 1901	Subject remained in bed until 4 P. M.	..	..	..	..	122	0	49	0

DATE.	REMARKS.	9 A. M.		10 A. M.		11 A. M.		12 M.	
		Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
4, IV, 1900	Diet largely carbohydrate	..	..	153	43	..	..	20	28
5, IV, 1900	Diet largely carbohydrate	..	..	123	9	..	..	56	40
8, IV, 1900	Subject remained in bed until 5.30 P. M.	..	..	..	..	..	..	80	13
4, XII, 1900	Diet largely proteid.	204	45	32	5	55	33	36	43
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	102	17	53	44	32	50	46	11
23, I, 1901	Additional hearty meal in bed at 3 A. M.	109	20	183	11	77	32	67	36
24, I, 1901	Arose at 5 A. M. Vigorous exercise all day.	400	24	211	13	17	29	50	53
1, II, 1901	Subject remained in bed until 4 P. M.	80	1	210	0	260	1	195	0

[9] A. Charrin. *Journal de Physiologie*, 1901, iii, p. 62.

TABLE II—*Continued.*

DATE.	REMARKS.	1 P. M.		2 P. M.		3 P. M.		4 P. M.	
		Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
4, IV, 1900	Diet largely carbohydrate	..	..	49	36	..	..	54	78
5, IV, 1900	Diet largely carbohydrate	..	..	53	42	..	..	168	24
8, IV, 1900	Subject remained in bed until 5.30 P. M.	..	..	93	13	..	..	365	0
4, XII, 1900	Diet largely proteid.	45	60	40	27	116	8	106	15
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	11	14	20	27	163	21	92	16
23, I, 1901	Additional hearty meal in bed at 3 A. M.	25	41	47	26	63	26	44	31
24, I, 1901	Arose at 5 A. M. Vigorous exercise all day.	50	13	54	25	24	32	38	41
1, II, 1901	Subject remained in bed until 4 P. M.	166	0	105	1	168	0	127	0

DATE.	REMARKS.	5 P. M.		6 P. M.		7 P. M.		8 P. M.	
		Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
4, IV, 1900	Diet largely carbohydrate	..	..	30	33	..	..	115	16
5, IV, 1900	Diet largely carbohydrate	..	..	34	74	..	..	62	8
8, IV, 1900	Subject remained in bed until 5.30 P. M.	..	..	97	1	..	..	68	49
4, XII, 1900	Diet largely proteid.	30	18	45	22	65	6	41	6
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	62	4	32	1	28	15	132	7
23, I, 1901	Additional hearty meal in bed at 3 A. M.	85	0	48	22	51	3	81	1
24, I, 1901	Arose at 5 A. M. Vigorous exercise all day.	45	26	37	15	52	18	80	5
1, II, 1901	Subject remained in bed until 4 P. M.	40	26	43	13	54	1	49	1

DATE.	REMARKS.	9 P. M.		10 P. M.		Day's Totals.	
		Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.	Vol. cc.	Proteid mgr.
4, IV, 1900	Diet largely carbohydrate	..	..	156	11	751	270
5, IV, 1900	Diet largely carbohydrate	..	..	141	10	777	235
8, IV, 1900	Subject remained in bed until 5.30 P. M.	..	..	219	43	1127	153
4, XII, 1900	Diet largely proteid.	165	1	231	0	1562	288
6, XII, 1900	Coffee at 8 A. M. No other food until 6 P. M.	40	24	50	7	985	268
23, I, 1901	Additional hearty meal in bed at 3 A. M.	114	0	37	88	1168	365
24, I, 1901	Arose at 5 A. M. Vigorous exercise all day.	91	14	80	7	1738	327
1, II, 1901	Subject remained in bed until 4 P. M.	122	25	147	9	1937	83

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